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EVALUATION OF SOME PHARMACOLOGICAL AND HISTOLOGICAL PROFILES OF THE EFFECT OF AN ALCOHOLIC EXTRACT OF THE DRIED ROOTS OF *MONDIA WHITEI* (PERIPLOCACEAE) ON ALBINO RATS, USING PROJECTED DOSES BASED ON THE FOLKLORIC USE OF THE PLANT

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, THE SCHOOL OF POST GRADUATE STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF PHILOSOPHY (REPRODUCTIVE BIOLOGY).

BY

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MARCH, 2005.

DECLARATION

I declare that I have wholly undertaken the study reported herein under supervision and that this work has not been submitted for any other degree.

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ABSTRACT

The alcoholic extract of the dried roots of *M. whitei* (reported to have aphrodisiac properties in local folklore) was evaluated for its pharmacological potential on the vas deferens of male rats and on the uterus of non-pregnant female albino rats. It was administered orally to the immature male rats to evaluate it androgenic or anti-androgenic activity in the testis and some accessory organs of the reproductive system.

No acute toxic symptoms were observed after an oral administration of up to 1052.16 mg/kg of *M. whitei* extract. Sub-acute and chronic toxicity studies also revealed no damage to vital organs like the kidney and liver.

Doses of ethanolic extract of *M. whitei* that were used were based on the prescribed folkloric intake of *M. whitei* dried roots. The dose required by an average 70 kg man was calculated and dose per body weight of each rat used extrapolated. Oral doses of 2.05, 4.11 and 8.22 mg/kg body weight administered for 3 weeks showed no androgenic or anti-androgenic effects with respect to the relative weights and histological profiles of sections of the testis, seminal vesicles, ventral prostate and epididymis. Increases in ventral prostate and epididymal weights of the extract-treated rats indicated no anti-androgenic activity. However, the reduction of the weight the of seminal vesicles after 7 days of treatment could indicate its inhibitory effect in this gland type and may be time dependent.

However, a combination of 2.05 mg/kg *M. whitei* and 1 mg/kg testosterone had a stimulatory effect on the weights of seminal vesicles and ventral prostate over the administration period of 21 days. There was also an increase in epididymal weight for this same dose level combination indicating possible androgenic activity of *M. whitei*. *M. whitei* administered at all dose levels was not anti-androgenic and therefore gave no indications of anti-fertility potential.

Experiments on the isolated rat vas deferens and rat uterus preparations indicate that *M. whitei* may have agonist activity on β-adrenoceptors. A relaxation effect on the uterine smooth muscle occurred.

It is safe to take *M. whitei* root extract even up to a dose of 1052.16 mg/kg with no visible toxic effect as well as causes no damage to the liver and kidneys at 8.22 mg/kg dose levels. *M. whitei* has no anti-androgenic effect on the male reproductive processes at the folkloric dose.

CHAPTER ONE

1.0 INTRODUCTION

The identification and utilization of plants useful to man from nature, commenced in prehistoric times. It has not yet been ascertained when herbal preparations were initially employed to cure ailments, but it can be assumed that their use began as early as the primitive man settled down to start cultivation and rearing of domestic animals (Bhattarai, 1992)

Knowledge of plant and animal extracts with desirable properties for various human ailments has evolved over the years and has been passed on to succeeding generations first orally and later in written documentation (Bedu-Addo, 1993). Experiments and trials were the two main ways through which man has learnt the various uses of plants. Such knowledge was initially shrouded in mystery and inevitably became associated with magic, alchemy and traditional religion and treatment of ailments became the preserve of witch doctors and fetish priests. Most western trained minds of today in many societies have yet to shed the idea that traditional healers work in the realm of magic that in no way relate to science (Bedu-Addo, 1993).

Traditional medicine, has been defined (by a 1978 group of experts from the WHO African Region, at a meeting over two decades ago in Brazzaville) as "The total sum of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation,

whether verbally or in writing". Traditional medicine is very complex and varies from country to country according to historical, economical and cultural development (Lo'i and Dung, 1991). Some practitioners of traditional medicine are therapists who treat their patients and obtain results just as orthodox medical practitioners do. Some even prescribe herbs and bandage wounds. They frequently have great knowledge of botanical lore and cannot only identify plants but also their properties. In many countries, the poor still depend on herbal folk medicine (Lo'i and Dung, 1991).

Traditional medicine especially the herbal folkloric medicine, has recently been receiving an increased interest all over the world (Bhattarai, 1992). The revival of the use of medicinal plants by developing countries and interest shown by WHO have led to intensified efforts on the documentation of ethno-medical data of medicinal plants. Most traditional healers keep no records and their information is passed on verbally, from generation to generation. Research has been geared towards finding scientific evidence for the claims as to the therapeutic efficacy of African herbs by traditional healers (Kaido *et al.*, 1997).

In developing countries, the treatment of diseases with herbal remedies is still popular (Shah *et al.*, 1991; Qureshi *et al.*, 1989). According to Bedu-Addo, (1993) (reporting from a WHO survey of 1967), between 60 %-90 % of patients in the third world are treated by traditional healers. For example, 60 % of patients in Indonesia, 65 % in Sri-Lanka, 65 % in Nepal, 70 % in Ghana, 80 % in India, 85 % in Burma and 90 % in Bangladesh receive traditional treatment.

Bedu-Addo in 1993, (quoting Nketsia, 1975) reported that a great deal of interest has been expressed in traditional medicine not only by those who believe in it, but by those who are fascinated by the thought that it may have something to offer to the practice of scientific medicine in certain countries such as Ghana. Her government attaches a lot of importance to traditional medicine, and a Centre for Scientific Research into Plant Medicine at Mampong, Akwapim has been established.

This development is however not peculiar to Ghana for there is a report of the existence of similar institutions also present in Nigeria, Uganda, Zaire and Tanzania (Bedu-Addo, 1993). Traditional medical practitioners enjoy good patronage in Nigeria. They are not only less expensive but also readily accessible. Akah and others in 1997 (reporting Akah and Nwambie, 1994) said that while it takes a considerable time for a patient to be seen to by an orthodox doctor (because of very high patient/doctor ratio), it takes considerably shorter time to see a traditional doctor. Furthermore, the "out of stock syndrome" (no drugs) in most hospitals does not exist in traditional medicine. Hence majority of the patients continue to hold traditional medicines in high esteem.

China, India, and other countries on the Asian subcontinent are also known to attach great importance to herbal medicine. China is the only country in the world where western medicine and traditional medicine are practiced alongside each other at every level of healthcare system. Traditional Chinese medicine has a unique theoretical and practical approach to the treatment of disease, which has developed over thousands of years.

Traditional treatments are said to include herbal remedies, acupuncture, acupressure and massage and moxibustion. They account for around 40 % of all healthcare delivered in China (Hesketh and Zhu, 1997).In Vietnam, although modern medicine succeeded in excluding traditional medicine until the restoration of national independence in 1945, the traditional medicine did survive with the development of the country. For thousands of years, Vietnamese people treated diseases with herbs and plants, which were, gathered from gardens and forests. Almost all the previous and present leaders were claimed to be cured with traditional medicine during the secret activity period of time, the recorded medical literature that now remains dates only after 10th century (Lo'i and Dung, 1991).

Bye and Dutton in 1991 (quoting from Cunningham, 1988), reported that despite westernization of the Zulus through urbanization and education, the belief in traditional remedies and healers remain. A dire shortage of western doctors in the rural areas, was estimated in 1982 to be in the ratio of one medical practitioner for every 17,500 peoples Bye and Dutton (1991) (quoting from Savage, 1985) further reported that rural inhabitants were forced to consult with traditional healers, if not by choice, then certainly by necessity. With a near exponential population growth rate, the demand for traditional remedies is increasing at an insatiable rate, placing severe strain on already depleted natural resources. Bedu-Addo (1993) (quoting Ayitey-Smith, 1989) reported that regardless of the highly advanced orthodox medicine, substantial amounts of medicinal plants are used directly for the treatment of ailments. In the USA, medicinal plants constitute about 25 % of all new and refilled prescriptions dispensed from community pharmacies. Between 1959 and 1974, American consumers paid 3 billion dollars for

drugs derived solely from medicinal plants, a similar trend is found in Britain and other European countries. Furthermore, many important drugs of modern medicine are still extracted from medicinal plants. About 50% of all drug preparations in the industrialized countries are derived from natural origin, and there is also the potential today for the industrial development of traditional herbal remedies and plant extracts for the treatment or prevention of diseases (Bedu-Addo, 1993).

It is in the recognition of this, that the World Health Organization (WHO) has called the attention of many countries to the ever increasing public interest to identify and exploit those aspects of traditional medicine that provide safe and effective remedies (Akah *et al.*, 1997).

Kaido and others in 1997, (quoting the work of Waller, 1993), stated that most countries with frequent usage of ethno-medical treatments have many traditional healers preparing herbal remedies or providing preparations to local populations. These healers could be used to great advantage if they were organized and encouraged to use only efficacious and safe herbal remedies while discouraging the use of ineffective and potentially toxic remedies. By performing scientific evaluations of efficacy for local ethno-medical preparations as well as organizing and disseminating scientific information to the local traditional healers, we could provide indigenous populations better access to efficacious drug treatment and an approved health status for those who cannot afford the benefits of modern medicines.

1.1 SUDDEN WARMTH OF THE DEVELOPED WORLD TO HERBAL/TRADITIONAL MEDICINE

The use of traditional medicine otherwise collectively termed as complementary and alternative medicine (CAM) has increased tremendously and is not new. Many have been used for hundreds and thousands of years, however, it may seem that there has been an explosion of use and awareness of these therapies in recent years. This is especially true for herbal medicine, which contain substances from the European herbal tradition, American Indian practices, African medicine, traditional Chinese medicine, Tibetan medicine, Ayurvedic medicine from India and other traditions (Kenny *et al.*, 2005).

A particular concern is the use of CAM (of which herbal medicines are the most frequently used) by patients without the knowledge of their physicians which may lead to complications. Inadequate literature and case reports from prestigious journals have caused the governments of developed countries to be interested in herbal medicine (Kenny *et al.*, 2005).

The use of herbal remedies is widespread across Europe. Complementary treatments are used by many doctors and other therapists throughout Europe of which one of the major forms again has been reported to be herbal medicine (Fisher, 1994). In UK, herbal remedies are increasingly being used by the general public on a self-selection basis to replace or complement conventional medicine. It was reported that in 1996, the market for licensed herbal remedies was estimated to be £38 million, representing over half of the total market for complementary remedies (Barnes *et al.*, 1998).

Relative popularities of these therapies vary widely. In the UK, practice is unregulated. Germany and some Scandinavian countries have intermediate systems (Fisher, 1994). Special licensing procedures for herbal remedies are already been carried out in Germany where regulatory evaluations of medicinal herbs have been laid in more than 300 monographs, and in France, more than 200 herbs have been listed as acceptable ingredients of phytomedicines (De Smet, 1995). The extensive use of herbal medicines in Germany is due to their availability (De Smet and Nolen, 1996). Strong herbal and folk traditions have also persisted throughout Eastern Europe (Fisher, 1994). The use of herbal medicines in Australia is widespread and has developed an integral approach to the herbal treatments that covers various non-western herbs (Drewand Myer, 1997). Growing numbers of people throughout the United States (40 % in 1998) were reported to use various forms of alternative therapies (LaFrance *et al.*, 2000). Research suggested that significant numbers of people are involved in various forms of alternative medicine (Austin, 1998) because they find these healthcare alternatives to be more congruent with their own values, beliefs and philosophical orientations towards life. The majority were found to use unconventional therapy for chronic as opposed to life-threatening, medical conditions in a research conducted to validate the use and cost of herbal medicine. The use of unconventional therapy in the USA is reported to be far higher than reported (Eisenberg *et al.*, 1993). But CAM use continues to increase. Studies found that the increase was from 33.8 % in 1990 to 42.1 % in 1997 (Kroesen *et al.*, 2002).

With the renewed interest from Western countries in herbal remedies, and the increasingly urgent need to develop new effective drugs, traditionally used medicinal plants are receiving attention of the pharmaceutical and scientific communities (Taylor *et al.*, 2001). The central Chinese government continues to have a policy for expansion of traditional medicine. It has become a source of great interest to the international research community. All western medical schools in China are reported to devote 10-15 % of the curriculum time to traditional Chinese medicine (Hesketh and Zhu, 1997). Some European countries have welcomed the idea of special herbal licensing, since the herbal wave sweeping over society is rising rather than falling. It offers opportunities to screen declared constituents, product quality and obtain information about the safe and correct use of herbal medicines (De Smet, 1995). Liberalisation of healthcare systems in Eastern Europe has led to an upsurge of interest in complementary therapies in the region. Some national governments in the west have funded research in this area. The Dutch government committed 1 million guilders a year, and in December 1992, the German Federal Ministry of Research and Technology advertised funding for research projects in complementary medicine. In 1994, the Committee on the Environment, Public Health and Consumer Protection of the European Parliament adopted a proposal on the status of complementary medicine. It also demanded an end to prosecutions of non-medically qualified practitioners in countries as such France and Spain and for a Pan-European system of regulation of non-medically qualified practitioners along the lines of the British Osteopaths Bill. Familiarisation with non-conventional medicine is compulsory in the German medical curriculum and is included in the undergraduate course in several French and Dutch medical schools (Fisher, 1994).

1.2 FLAWS IN THE USE OF TRADITIONAL MEDICINE

The question, whether the use of traditional medicines can entail a health risk, is a rhetorical one. Plant medicine has its fair share of problems; it is not exactly problem-free. A number of publications both in the scientific and the lay press exist on medicinal use of indigenous plants. It is well established that all sorts of vegetable, animal and mineral remedies used in a traditional setting are capable of producing serious adverse reactions. De Smet (1991), quoting from an anonymous source (1988), and also from an authoritative review published fifteen years ago by WHO (1989) listed over 280 species of plants containing hepatotoxic pyrrolizidine alkaloids. Also quoting Penso (1983), De Smet (1991) reported that more than 60 of these are listed as medicinal plants in the *Index Plantarium Medicinalium*.

It is generally known that herbs in their crude form may have both curative and toxic effects on man. The indications for the use of various medicinal plants are usually not well defined and often the plants may be used for several unrelated disorders. Also poisoning occurs because of misidentification of a plant or ones with unknown or ignored toxicity of a correctly identified plant. All these, coupled with the apparent lack of standardized dosage and effective methods of preservation have made traditional herbal medicine a target of severe criticisms (Bedu-Addo, 1993). The inappropriate use of herbs has resulted in numerous fatalities, invariably in children, in all parts of the world. It is recounted in South Africa that on one occasion, a child was admitted to a rural hospital suffering from hepatitis, treated intensively, recovered fully and discharged. A week later the child died as a consequence of the administration of the herbal remedy *Impila* used in

treatment (Bye and Dutton, 1991). *Impila* is of multifunctional use to the people of South Africa. According to Bhoola (1983) as reported by Bye and Dutton in 1991, scientific interest in this herb was increased when in the mid 70's a high incidence of centrilobular liver necrosis accompanied by renal necrosis accounted for 2 % of all deaths at the King Edward VIII Hospital. Again, quoting from the work of Wainwright *et al.*, (1977) and Bhoola (1983), Bye *et al.*, (1991) reported that *Impila*, scientifically known as *Callilepis laureola* was identified to be the primary causative agent for some two hundred and sixty deaths, a third being in children of less than five years of age.

De Smet quoted from a 1985 journal, *Gut* that published a report about four young Chinese women who were using herbal tea as treatment for psoriasis. It was that they developed hepatomegaly and three of them discontinued the tea and made a recovery. The fourth woman continued with intake of the tea and her condition progressively deteriorated and died from hepatic failure. Plants grown from seeds encountered in the tea were botanically identified as *Heliotropium lasiocarpum* (De Smet, 1991). In December 1983, the Journal of the American Medical Association reported on a fifteen-month-old child and his three year old sibling, who had been treated with multiple doses of azarcon, locally known as *empacho* which is used as a folk remedy for gastrointestinal and abdominal complaints. The three year-old died and the death was suspected to be azarcon-induced encephalopathy (De Smet, 1991). Herbal toxicity has also been reported in Zimbabwe. Indigenous natural drugs are are said to be commonly used because modern life-saving are beyond the reach of nearly 85 % of the population. But these natural drugs have been reported to have caused a number of poisoning cases. Four

hospitals from 1971 to 1982 have shown several people were poisoned with herbal remedies (Nyazema, 1986).

Many medicinal plants used in ethno-medical practices are completely unknown or only little known to the scientific world. The pharmacological activities of most of these plants remain to be studied (Subramoniam *et al.*, 1997). Aside their pharmacological properties not known, many contain highly toxic principles. Undoubtedly the use of some of these herbal remedies has had fatal consequences (Bye and Dutton, 1991). Furthermore, the traditional medical practice is rife with many quacks and the claims made by many are over enthusiastic. Little do they know and accept that these curing herbal medicines have side effects and how serious these adverse reactions produced can be. A typical example is as in China, where male infertility was caused from ignorantly eating food that contained crude cotton seed, because the seed was then not known to contain gossypol which affects male fertility. The debarked root of *Tripterygium wilfardii* is a traditional Chinese medicine used for the treatment of several diseases, but which was later found to also induce antifertility in male mice (Pei-Gen and Nai-Gong, 1991).

There is therefore the need to investigate scientifically the reported therapeutic properties of plants, their toxicity, as recommended by the 1967 WHO scientific group and standardization of dosage forms (Bedu- Addo, 1993). It is also essential, however, that the traditional drug therapies are submitted to an appropriate benefit or risk analysis (De Smet, 1991). In the KwaZulu region, for instance, by law, according to the Zulu Law,

Act 6 of 1981, all herbalists are required to be registered; and a prerequisite being that the person is 'skilled in herbalism' (Bye *et al.*, 1991).

1.3 APHRODISIACS

An aphrodisiac is any form of stimulant thought to arouse sexual excitement. They may be classified in two groups: (1) psychological (visual, tactile, olfactory, and aural) and (2) internal (stemming from food, alcoholic drinks, drugs, love potions, medical preparations). Of various foods to which aphrodisiac powers are traditionally attributed, fish, vegetables and spices have been the most popular throughout history. And throughout history, people have searched for the "sure fire" aphrodisiac, but that search has largely been unsuccessful, as there is no known substance that works well as an aphrodisiac. There is a myth that says that foods that resemble sexual organs have sexual powers. Hyde and DeLamater (1997) quoting the work of MacDonald in 1961,to which Neiger, 1968, debunked said oysters are thought to have such powers because of the resemblance to the testes. As quoted by Hyde and and DeLamater (1997) again, Neiger (1968) reported that oysters actually do not contain any substance that can in any way influence sexual functioning.

A number of botanical products are purported to enhance sexual function and act as aphrodisiacs. A cross sectional study of women in Malawi reported the use of traditional substances to tighten the vagina before coitus. It has been reported that many women in Southern Africa willingly insert herbal aphrodisiacs before coitus (Cohen *et al.*, 1996). They insert household detergents and antiseptics, and this is claimed to increase friction.

In Zaire, women are reported to use leaves and powders to heighten sexual power (Baleta, 1998).

Brassia rapa, Prunus amygdalus and *Zingiber officinale* have been used as aphrodisiacs in Arab medicine (Qureshi *et al.*, 1989). *Withania somnifera (L)* is a perennial or semiwood shrub growing in Sri Lanka. According to traditional medicine of Sri Lanka, India and Nepal, the roots of *W. somnifera* possess aphrodisiac activity which has not been scientifically documented (Hayperuma *et al.*, 2002). For more than 70 years *Yohimbe* has been used as a treatment for male and female sexual difficulties. It has enjoyed the reputation as an aphrodisiac although no effect on sexual drive in humans has been adequately demonstrated (Riley, 1994). Ginseng, the root of the perennial herbs of which *Panex ginseng* and *Panex quinquefolium* which contain a series of tetracyclic triterpernoid saponins as active ingredients is believed to enhance sexual performance (Nocerino *et al.*, 2000). *Damiana (Tunera diffusa)* is reported to be an aphrodisiac, stimulant, mood elevator, and "tonic", and has been in use in the United States since 1874. It is said to have testosterogenic activity which may account for its traditional use by the Mayan people of Central America for enhancing sexual function in men and women (Cohen *et al.*, 1996). Quoting from research done by Puri, 1971, Islam *et al.*, (1991) also reported that Hindu medicine still claims *Aristolochia indica, Crocus sativus, Apinina galanga and Allium cepa* as potent aphrodisiacs. Al-Bekairi *et al.*, (1991) to support the acclaimed aphrodisiac potentials of *A. cepa*, did further research on this herb. Gauthaman and others (2003) reported that apart from its claims for improvement of sexual functions in men, sexual behaviour and intracavernous pressure measurements to scientifically validate the aphrodisiac nature of *Tribulus terrestris* (TT) confirmed that

the pro-erectile aphrodisiac property of TT could be due to increase in androgen and subsequent release of nitric oxide from the nerve endings innervating the corpus cavernosum.

Hyde and DeLamater (1997) quoting Teberner, 1985 reported that, unfortunately some of these substances that are thought to enhance sexual functioning are quite dangerous. *Cantharides* known as "Spanish fly", is said to be poisonous because the amyl nitrate it contains can cause death. He further on stated that some of the well known aphrodisiacs contain substances that act differently during sexual functioning or just simply believing that something will be arousing can itself be arousing. For instance, the effect of the popularly known "poppers" among some homosexuals and heterosexuals is actually the amyl nitrate it contains which just relaxes the sphincter muscle of the anus and probably acts just by dilating the blood vessels in the genitals during orgasms (Hyde and DeLamater, 1997).

1.4 THE OBJECTIVE

M. whitei is a widely used medicinal plant by the traditional healers around the continent of Africa. It has been catalogued by the Center for Research into Plant Medicine at Mampong-Akwapim and is on sale on the Ghanaian market. Since the plant is purported to be an aphrodisiac, as well as being able to increase sperm count, and generally function to increase male fertility, the main objective of this research is to attempt to evaluate the validity of, or otherwise of some of the claims alluded to *M.whitei* using *Rattus novergicus* (the albino rat) as a model organism.

The specific objectives are to determine (based on projected human equivalent doses):

1. The toxicity level of an alcoholic extract of *M. whitei* in albino rats
2. The effect of *M. whitei* on spermatogenesis, the tissues of the testes
3. Whether *M. whitei* has any effects on some male accessory and reproductive structures.

1.5 JUSTIFICATION

Toxicity tests were performed because *M. whitei* has wide folkloric use by the general public. Thus, there will be an assessment to establish whether the extract is harmful to any soft organs of the albino rat, especially the liver (hepatotoxicity) and kidneys (nephrotoxicity) or that it causes any other general systematic harm. The similarity in the physiology of man and the rat should allow us to extrapolate and therefore to raise some caution regarding its use by man.

Secondly, there was a need to obtain some scientific validation regarding the assertion that *M. whitei* improves spermatogenesis. Thus histological sections of the testes were examined to assess improvement or otherwise the stages of sperm development. This should allow us to indirectly speculate the improvement in the secretion of gonadohypophyseal hormones. Positive findings along these lines should give some credibility to the folkloric assertion that it improves male fertility.

Lastly the effect of *M. whitei* on isolated rat uterus was examined to assess whether it is a muscle relaxant.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 FOLKLORIC USE OF PLANT AND NATURAL PREPARATIONS FOR IMPROVING FERTILITY AND SEX DRIVE

Despite the increasing availability of effective conventional medical treatments, plant-derived and herbal remedies continue to provide a popular alternative for men and women seeking to improve their sex life (Rowland and Tai, 2003). Natural products have been reported to become an increasingly popular treatment option for patients who have been dissatisfied with standard Western pharmacotherapy (Cohen *et al.*, 1996).

"Potency wood", also known as *Muira puama* is a shrub that has been used in South American medicine for treating decreased libido. It was reported that in a French clinical study in 1990, *M. puama* extract improved libido in 62 % of patients with lack of sexual desire (Cohen *et al.*, 1996). Ernst (1999) reported that several plant-based medicines are useful additions to therapeutic repertoire for the elderly of which *Yohimbe* has been shown to be effective for erectile dysfunction. Anecdotal reports in the scientific literature suggested that ginseng can improve sexual dysfunction such as anorgasmia and decreases libido. Chinese root *Panax* and North American *Panax quinquefolium* contain ginsenosides that facilitate the release of nitric oxide, a step in events leading to erection. This property was reported to be promising for women since the biochemical process of erection is the same in the clitoris (Cohen *et al.*, 1996). The FDA has this Chinese herbal on its safe list (Leak, 2005). "Mkombelo", a herbal medicine which is scientifically known as *Mondia whitei* is believed strongly and is popularly linked to the management

of impotence problems in Kenya. It is also said to help in the management of sexually transmitted diseases because of its antibiotic properties. The believe in the herb that it enhances desire for sex together with its ability to manage STDs seems to be the cause why it has been depleted very fast in Kenya (www.nationaudio.com). Benign prostatic hyperplasia (BPH) is a condition that leads to male sexual dysfunction. The pharmacological use of plants and herbs for the treatment of BPH has been growing steadily. Extracts from fruits of *Seronea sepens (*Saw palmetto*)* and the roots of *Urtica dioica* (stinging nettle) are popular for the treatment of BPH (Koch, 2001). *Pygenum africanum* was reported as one of the phytotherapeutic agents available for the treatment of BPH (Wilt *et al.*, 2002).

"Ashwagandha", "Rhodiola", *Cordyceps* fungus, "Shilajit" or "Mummio", "Smilax" and "Suma" (*Ectoystrone*) are all herbs that are reported to be currently used to enhance physical sexual performance. *Rhamnus frangula* has been tested in human clinical trials and is said to improve strength, endurance time and feelings of well-being (Luke, 2000).

An alternative to chemical medication in the treatment of sexual dysfunction in healthy women was investigated with a unique herbal formulation of *M. puama* and *Ginkgo biloba* (Herbal vX) in 202 healthy women with low sex drive. Significant improvements in frequency of sexual desires, sexual intercourse and fantasies as well as the ability to reach orgasm supported the strong anecdotal evidence for the benefits of Herbal vX on female sex drive (Waynberg and Brewer, 2000). Some herb and plant extracts can be used for female antidepressant-induced sexual dysfunction, sexual enhancement and

menopause. Black cohosh (*Cimicifuga racemosa*) has been used for decades in Germany, USA and Australia. It has been reported to allcviate menopausal symptoms and originally used by Native Americans. *Vitex agnus castus* (chasteberry) is reported not to reduce libido in menopausal women as thought for women in the childbearing age. Wild yam preparations available in creams and oils, contain natural progesterone which is also reported to have won the favour of many per menopausal and postmenopausal symptoms, it may improve sexual desire (Cohen *et al.*, 1996).

2.2 EFFECT OF SOME HERBAL MEDICINAL PLANTS ON REPRODUCTIVE SYSTEMS AND FERTILITY

Dealing with infertility can challenge one's patience, budget and even one's marriage; since evaluating, testing and treating infertility can be very expensive and stressful and may even come with side effects (Infertility, www.planetrx.com). It is due to this that a lot of research is being done on medicinal plants that are claimed to have some positive effects on the functioning of reproductive systems. These therapeutic and medicinal uses are now being mentioned in literature (Adhikary *et al.*, 1990) and pharmacological investigations reported.

2.2.1 PLANT PREPARATIONS THAT PROMOTE FERTILITY

A variety of plants have been used as sex stimulants in the traditional medicine systems of various countries (Islam *et al.*, 1991). *Yohimbe* is derived from the bark of the *Yohimbe* tree (*Pausinystalia yohimbe*) and its primary action is to increase blood flow to erectile tissue. It is said to have been approved by The US Food and Drugs

Administration (FDA) for treating male erectile dysfunction (Cohen *et al.*, 1996). Islam and others in 1991 (quoting Messegne, 1973) reported that in European countries, a cream used for vaginal douches to increase sexual desire in frigid women is prepared from *Chelidonium majus*. An aqueous extract of *Cynomorium coccineum* administered to ten mature male wistar rats, at a dose of 47 mg/100 kg body weight for 14 consecutive days induced increase in sperm count, improved the percentage of live sperm their motility as well as decreased the number of abnormal sperm. Histologically, increased spermatogeneis was reported (Abd El-Rahaman *et al.*, 1999).

For centuries, Arabs have made use of herbal drugs supposedly improving sexual performance and increasing libido. Islam *et al.*, (1991) (quoting the work of Puri, 1971) mentioned that the pollen grains of dates (*Phoenix dactylifera*) and seeds of hermala (*Peganum harmala*) are used in Egypt to restore sexual potency. In Saudi Arabia, several native plants are used in the belief to increase sexual desire and performance. Islam and others, (1991) (reporting the work of Baitar, 1871) said the roots of *Salvia haemotodes* commonly known as red sage is often prescribed for the treatment of sexual disorders. Islam *et al.*, (1991), (quoting Said, 1969) further reported that *S. haemotodes* aside its use as an aphrodisiac, is also used for treatment of premature ejaculation of semen. Islam and others in 1991, based on scientific observation and findings supported the use of this plant as a sex stimulant and for the treatment of premature ejaculation in traditional medicine. *Tricus zeylanicus* is unique in possessing many pharmacological activities including its aphrodisiac action (Subramoniam *et al.*, 1997). *Ruta chalepensis* failed to produce any spermatotoxic effect in use (Shah *et al.*, 1991).

Traditional or indigenous systems of medicines have persisted for many centuries, even where modern health care is readily available. Most pregnant women who use herbal remedies do so to relieve pregnancy-related stress while the remainder use it as a consequence of beliefs, though possibly superstitious, or as a result of circumstantial constraints or combinations of these, as reported by Kaido and others (1997) (on the findings of Mbura *et al.*, 1985). Many black South African women use traditional herbal remedies as antenatal medications or to augment labour and expel the placenta. A literature survey conducted by Veale and others (1992), as again reported by Kaido *et al.*, 1997 indicated that at least 57 different plants are used by these women. These remedies are known as'Isihlambezo','Imbelikisane' or 'Inembe' and their three basic plant constituents that have been pharmacologically examined are *Agapanthus africanus, Pentanisia prunelloides* and *Gunnera perpensa L* respectively. Mpondo women grow *Agapanthus africanus* in water, and drink some of the water, night and morning, from the fourth to the fifth month of pregnancy, with three objectives; 1) to ensure a healthy child, 2) to ensure the child will not develop bowel trouble and 3) to ensure that the placenta will be delivered without difficulty. Kaido and others (1997) (reporting work done by Batten and Bokelmann, 1967) stated that *Pentansia prunelloides* is known in Zulu and Xhosa as 'Icimamlilo' and its root is believed to anchor the placenta and therefore prevent miscarriages both in man and animals. Kaido and others in 1997, (quoting Bryant, 1966) stated that in order to facilitate the due expulsion of the after birth and proper cleaning of the womb, the Zulu and southern Sotho drink a concoction of the root of *Gunnera perpensa*. Veale and others (1992) reported *A. africanus, P. prunelloides* and *G. perpensa* to be the ingredients of 'Inembe'.

2.2.2 PLANT PREPARATIONS THAT HINDER FERTILITY

The human race has been facing increasingly many upward population explosions, and thus much attention has been paid by many international bodies to one of the key measures: birth control (Pei-Gen and Nai-Gong, 1991). In spite of several major problems they face, traditional medicines have gained new impetus in a lot of countries, and their use in controlling fertility in human is seriously being considered. In India, for example, emphasis has been placed on plant sources; as a number of useful herbal drugs on fertility control have been developed over the past two decades (Prakash *et al.*, 1991). China one of the most densely populated regions with approximately one fifth of the world population has been making great efforts in controlling her population size. One of the efforts is to look into the practicability of employing Chinese herbal medicine for fertility control (Pei-Gen and Nai-Gong, 1991). In 1988, reported in the *Compendium of Chinese Medica*, were a total of 288 drugs described with applications associated with fertility control; of which 27 are abortifacient, 97 as emmenagogue, 44 as uterine stimulant and 60 as contraindicated in pregnancy (Pei-Gen and Nai-Gong, 1991). Pei-Gen and Nai-Gong (1991) (quoted Wu and others, 1988), reported that, a collection of ethno-pharmacological data from Chinese traditional medicine revealed that 817 species and varieties of Chinese medicinal plants are ascribed to indications related to fertility control.

Although hundreds of medicinal plants have been reported to possess antifertility activity, not many products have been developed as a potent antifertility agent from these sources. Moreover, the mechanisms of action of many potent extracts are not fully understood

(Prakash *et al.*, 1991). To be able to provide a lead in developing new antifertility drugs from natural products, WHO has set up a task force on plants for fertility regulation, the strategic plan of which is to identify novel drug prototypes found in plants which have been alleged to have fertility regulating properties. Pei-Gen and Nai-Gong (1991) (quoted the work of Griffin, 1988), stated that the compounds being sought after in particular are those orally active, non-steroidal, non-estrogenic, safe and effective types for the prevention or disruption of implantation in women and those that will inhibit spermatogenesis or interfere with sperm maturation in men. In spite of considerable development in contraceptive technology, search for male antifertility agent in plants continues to be a potential area of investigation. Antispermatogenic activity has been reported in a few plants (Choudhary *et al.*, 1991).

Anti-fertility effects of ethanolic leaf extracts of *Alstonia scholaris*, *Cleistanthus collinus* and *Terminalia bellirica* and root extract of *Murraya paniculata* were observed in male albino rats after oral administration. A significant reduction in sex desire (libido) was also observed in males treated with *C. collinus* (Choudhary *et al.*, 1991). *Striga orobanchiodes* was reported to affect male fertility by exhibiting an antiandrogenic activity (Hiremath *et al.*, 1997). Leaves of *Anacardium occidentale* have definite effects on male reproductive function. It has an antifertility effect due to the effect of β-sitosterol as one of its chemical constituents. High dose of β-sitosterol that caused an observed decrease in testis and accessory organs may be due to the intrinsic oestrogenic/ antiandrogenic activity of the compound (Malini and Vanithakumari, 1991). Malini and Vanithakumari in 1991 (quoting Orgebin *et al.*, 1983) reported that male accessory sex

organs are known to respond to oestrogen treatment but responses varied according to age, species, hormonal status and gland type and could also vary with the types of tissue within a single gland. Malini and Vanithakumari (1991) (further quoted the work of Mann and Lutwak, 1951) indicated that an oestrogenic hormone which inhibits male gland secretion if administered in large doses may have a definite stimulating effect when applied in small quantities. Chronic oral administration of the crude alcoholic extract of the stalk of the leaf of *Piper betle (Linn)* showed antifertility effect and it could be concluded that the plausible cause of infertility in male rats was due to inhibition of steroidegenesis (Adhikary *et al.*, 1990). After 48 days of dosing with an extract of *Andrographis paniculata*, a decrease in sperm number and motility and increase in morphological abnormalities were seen in the spermatozoa. Ethanolic extract of *Colebroo-kia oppositifolia* at a dose level of 100 and 200 mg/kg/d for 8 to 10 weeks did not cause any weight loss, but significantly decreased the weight of the testes and epididymis and notably reduced sperm count. Petroleum ether extract of the leaves of *Mentha arvensis* showed antifertility effects in male mice when dosed orally for 60 days. A significant decrease in testicular weight and caudal sperm count was observed (Nivsarkar *et al.*, 2002). *Ferula sinaica* may be one of the plants that have some antioxytocic potential (Aqel *et al.*, 1991).

Various extracts of *Crotalaria juncea (Linn)* seeds arrested spermatogenesis and are likely to have an antiandrogenic activity. Adult male rats were gavaged the petroleum ether, benzene and ethanol extracts of *C. juncea* seeds, 25 mg$(100\ mg)^{-1}day^{-1}$ for 30 days. There was an observed reduction in spermatogenic elements which could have indicated

lowered ability of FSH and LH/ICSH which are known to be essential for the initiation and maintenance of spermatogenesis. Testicular and accessory organ weights diminished and seminiferous tubular lumen, devoid of spermatozoa (Vijaykumar *et al.*, 2004).

Naseem and others (1998) experimented on the antispermatogenic and androgenic activities of *Momordica charantia* in albino rats. They tested petroleum ether, benzene and alcoholic extracts of *M. charantia* seeds at a dose level of 25 mg/100 g body weight for 35 days. These extracts with the alcoholic extract more potent showed antispermatogenic, antisteroidogenic and androgenic activities. Weights of some accessory organs increased indicating androgenic activity of *M. charantia*. *Achillea*, a medicinal herb, native to Europe, North America, Northern Asia and some parts of Iran is not only popular in its antihemorrhagic and analgesic properties, but has some of its varieties putting an antispermatogenic arrest on spermatogenesis. A hydroalcoholic extract of *Achillea. santolina* at 300 mg/kg/day for 20 days had antispermatogenic effect similar to that of *Achillea. millefolium* on mice. There was no reported significant change in body weight, but a reduction in testis weight was observed coupled with some alterations such as disorganized reduced germ epithelium, degenerated and necrotic cells, indicating an inhibitory effect of *A. santolina* on mice spermatogenesis at 300 mg/kg, administered intraperitoneally for 20 days. *A. millefolium* was also reported to have antispermatogenic and degenerative changes in the mice testes (Golalipour *et al.*, 2003).

Oestrogens seem to be involved in the regulation of testicular activity. It has been hypothesized that the block of spermatogenesis and the complete regression of the

epididymis and the other secondary sexual characteristics in autumn of the *Podarcis sicula* lizard might be due to high oestrogen levels. Research done by Cardone and others (2002) proposed that the failure of spermatogenesis in autumn might really be due to the high oestrogen levels. Fadrozole, a non-steroidal inhibitor of aromatase, the enzyme involved in the aromatization of androgens to oestrogens, when administered at a higher dose caused decreased oestrogen levels resulting in release of sperm into large lumen of seminiferous tubules with a more developed epididymis (Cardone *et al.*, 2002). Seed extracts of *Vitex negundo* are reported to interfere with male reproductive function without producing adverse toxicity in other vital organs. A partially purified flavonoid rich extract from these seeds at a dose of >15 mg/rat/day after 15 days of treatment caused a decrease in weight of all the major accessory sex organs. The drop in weight was also reflected in disturbed tissue biochemistry and indices of accessory sex organ function diminished. Sperm numbers dwindled and slackness in their motility observed, factors that impede fertility (Das *et al.*, 2004).

Lohiya and others (1999) confirmed that benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* possess reversible male contraceptive potential and the effects appear to be mediated through the testes. Administration of 50 mg/animal/day for 150 days in adult male rabbits resulted in uniform azoospermia for 15 days of treatment which was maintained for the 150-day observation period. The libido of the treated animals was normal and the fertility rate zero with complete normalcy of parameters observed 60 days following withdrawal of treatment (Lohiya *et al.*, 1999). Pongomia oil has also been reported to have strong spermicidal activity (Bandivdekar and

Moodbidiri, 2002). A refined extract from the root of *Tripterygium wilfordii*, a perennial twining vine found in Southern China, has been demonstrated to exert a powerful antifertility effect in both rats and human males. Fertility appeared though to be reversible after cessation of treatment. Dramatic decreases in density and motility of spermatozoa from the cauda epididymis without apparent testicular damage was reported by Qian in 1988.

Crotalaria juncea (Linn) commonly known as Sunn hemp cultivated throughout India was indicated by Indian Ayurvedic medicine also to have emmenagogue and abortifacient activities (Vijaykumar *et al.*, 2004). *A. millefolium* is indicated to be a contraceptive, abortifacient and emmenagogue used by the people from Northern Europe and North America (Golalipour *et al.*, 2003). Shah and others in 1991 (quoting work done by Rao *et al.*, 1988) stated that *Ruta graveolens* has been reported to possess abortifacient and inflammatory properties. Medeiros *et al* (2000) (quoting work done by Sundaresson, 1942), said that senecionine, a pyrrolizidine alkaloid from *Seneciojacobea spp*, crosses the placenta drastically affecting the foetus. *Murraya paniculata* exhibited some abortifacient effect (Choudhary *et al.*, 1991). The result of a WHO joint project showed that the screeningof some 8 plants demonstrated the activity of pregnancy termination in animals. One of such plants was *Marsdenia koi*. Pseudolaric acid B that was isolated from the root bark of *Pseudolarix kaempferi* does not exhibit any anti-implantation activity, but could result in termination of early pregnancy (Pei-Gen and Nai-Gong, 1991). *Cannabis sativa* has been studied and reported to adversely affect

pregnancy and foetal development. When given with alcohol, it increased foetotoxicity (Agnihotri *et al.*, 1992).

A decline in implantation sites and litter size was found in females mated by males treated with leaf extracts of *Alstonia scholaris, Cleistanthus collinus*, and *Terminalia bellirica*. This may be due to the direct effect of the leaf extracts in the female genital tract with semen from the treated male rats which caused the low luteal activity (Choudary *et al.*, 1991). Hiremath and others (1997) reported that *Striga lutea, S. orobanchioides* and *S. densiflora* have shown significant anti-implantation and oestrogenic activity in female rats. Prakash and others (1991b) reported that a hexane extract of the aerial parts of *Ferula jaeschkeana* inhibits implantation. The flowers of *Gardenia jasminoides* have been used in Chinese folk medicine for birth control. It was found out that the ethyl acetate extract of the flowers has a significant action of pregnancy termination in rats. Two cycloartene triterpenoids, gardenic acid (V) and gardenolic acid B (VI) were also isolated and found active. They damage the decidual cells of early pregnant women (Pei-Gen and Nai-Gong, 1991). *Myrica cerifera* commonly known as bayberry found all over the drier regions of Continental United States has a long history of use in North America. Bayberry has been reported to be used to stimulate sluggish contractions during labour while at the same time preventing or arresting post-partum hemorrhaging (Matsuda *et al.*, 2001).

The oil fraction, extracted mechanically from the seeds of *Azadirachata indica* (neem) is considered to be the most effective part to exhibit significant antifertility effect when

administered subcutaneously or intravaginally (Prakash *et al.*, 1991a). It has been reported that *Vicoa indica* is used as a folkloric female antifertility agent in Madhyapradesh, India (Gopal *et al.*, 1992). *Phyllanthus amarus* is said to have a definite contraceptive effect in female mice. Upon withdrawal of the extract, after 45 days of feeding, these antifertility effects were reversible (Mandava and Kuvian, 2001).

In North Yemen, it was reported that infant and childhood mortality rates were found to be equal or exceeding 50 % till 1976, when the reproductive toxicity rates of *Catha edulis* or khat was evaluated. Khat chewers were found to claim varying effects on their sex life. In females, Bekairi and others (1991) found out that methanolic extract of khat leaves upon oral feeding induced significant foetal loss, abnormalities in growth of foetuses and it is also induced in abortifacient activity and anti-oestrogenic effect. The post pregnancy treatment inhibited the implantation rates up to 40 %. It is most reportcd that spermatogenic activity, semen output sperm concentration and sperm abnormalities were decreased with Khat treatment at certain doses during a particular time frame. Prolonged exposure resulted in the induction of total and partial sterility males (Bekairi *et al.*, 1991)

2.3 *MONDIA WHITEI*

2.3.1 DESCRIPTION AND GEOGRAPHICAL LOCATION

The two species of *Mondia* are native to tropical Africa with *M. whitei* occurring in southern Africa, a liana in the tree canopy of riverine and swamp forest in the KwaZulu-Natal (Victor *et al.*, 2000). *M. whitei* has large tuberous rootstock, stems puberlous,

glabrescent with persistent lignascent stipular frills at nodes and has large prominent lenticels. Leaves are rounded, petiolate, 17 cm long and 15 cm broad. Flowers of *Mondia* have lateral pendiculate panicles up to 15 cm long and 10 cm across (Hutchinson and Dalziel, 1963). This 3-6m vigorous woody soft climber that originates from tropical Africa can also be found in the wet bushes and savanna areas of Kenya, in the Casamance region of Senegal, Cameroun, and also in the Congo (Watson and Dallwitz, 1999).

It used to be common throughout the great belt of forest across mid Africa. Due to the gradual and progressive diminishing of the content of the forest in Kenya for example, it is now confined almost completely to the Kakamega forest, the only remaining tropical rain forest (www.essential oils.com). But fortunately this shrub grows throughout the Malawian country (www.mapinc.org). *M. whitei* has also been recorded in the Durban area of South Africa. It is reported to be one of the plants used by the population of some developing countries as a primary source of healthcare (Cunningham, 1993).

2.3.2 CHEMICAL COMPOSITION OF THE ROOT OF *M. WHITEI*

Roots of *M. whitei* contain sugars like glucose and sucrose, which are known to be as the main types of sugars in a phenolic glycoside isolated from the roots. Roots contain 2-hyroxy-4-methoxybenzaldehyde and 3-hydroxy-4- methoxybenzaldehyde also known as isovanillin (Koorbanally *et al.*, 2000).These compounds are responsible for the characteristic sweet aromatic fragrance of *M. whitei* root back. The chemical properties of 2-hyroxy-4-methoxybenzaldehyde are responsible for the taste modifying character (Mukonyi and Ndiege, 2001). *M. whitei* also contains tyrosinase, and the general plant

contains zinc, iron, calcium, and vitamins A, K, D and E (www.essential oils.com). Additionally, its roots are a source of magnesium and protein (www.mnh.si.edu).

2.3.3 FOLKLORIC USES OF *M. WHITEI*

M. whitei is used in the Kinshasa region in, Zaire as a therapy for breathing difficulties in children, especially against asthma. A concoction of the flower can be drunk or the root bark chewed with food to achieve this effect. The flowers can also be held over hot flaming coals and emanating vapour inhaled. *Mondia* roots are also used as remedies for stomach ailments by the Bondei people in Tanzania. The Masai of Kenya boil the roots with some fatty beef or lard and the extract drunk as a laxative. In Malawi, the powdered root is used as a remedy for headaches; stomach upsets as well as diarrhoea. The Shona people of Zimbabwe also use the root back extract as a remedy for constipation, loss of appetite in cases of *Anorexia nervosa* and bilharziasis. *M.whitei* is used for making fishing lines in East Africa (Lind *et al.*, 1974). The Zulus use the plant medicinally to make a beverage similar to ginger beer (Watson and Dallwitz, 1999). Also during the 19th and 20th century the roots of *M.whitei* was among the African flora studied by British colonial botanists as a potential for commercial exploitation. They reported that these roots are traditionally used by the Zulu's as flavouring for soft drinks (Cunningham *et al.*, 1997). According to Mukonyi Kawaka, a phytochemist at the Kenya Forestry Research Institute, *M.whitei* has enormous nutritional value and on going studies show it may be effective against up to 50 diseases. He also claims it makes the skin lighter if ingested (www.essentialoils.com). The climbing herb is used as (I) an appetizer, (2) to enhance milk production in lactating mothers, (3) for the management of diabetes and

hypertension, (4) for managing allergy problems and (5) for flavouring foods (www.nationaudio.com). It is also claimed that when farmers in Western Kenya feed *Mondia* roots to their lactating cows there is an increase in milk production, but Wekesa and others reported that responses to these roots were quite low compared to other conventional feed supplements (www.cannabisculture.com).

Roots, leaves and stem of the *M. whitei* plant are used widely by several African tribesmen especially the traditional healers. It has been reported to be highly considered as an aphrodisiac and popularly linked to the management of impotence problems (www.nationaudio.com). The Gambaga Kara natives in the South Western region of the Central African Republic use the pulverized form of the roots to enhance sperm production. In Mozambique the powdered root is taken in with some soup or beer for its aphrodisiac purposes. In 1998, when Viagra became available in Kenya a flurry of letters appeared in the Daily Nation, the writers of who were all from the Luhya and Luo tribes of western Kenya claimed that they had their own Viagra. The wild ivory coloured vine root, *M. whitei* known as 'Ogombo' in Dholuo and 'Mkombele' in Kiluhya was according to these writers, a renowned sexual stimulant for men (www.essentialoils.com). In Malawi Mponda (2002) a writer of the Mail and Guardian, a South African paper, reported that *M. whitei* which is known as 'Gondolosi' is a well recommended aphrodisiac and could be termed as Malawis' homegrown Viagra. Augustine Saudeni, a Malawian expert in traditional healing plants said that it is up to the government to approve its export even though Joe Manduwa, an agricultural expert reported that the traditional healers were secretive about the herb, fearing scientists would

reap financial rewards from the knowledge (Mponda, 2002). However, this same herb was found to have a partial infertility effect after about a use period of 55 days of continuous use at a dose of 400 mg/kg/day. A recovery period though resulted in normal spermatogenesis and fertility, suggesting reversible antispermatogenic effects of the plant (Watcho *et al*., 2001). In South Cameroun, the Bafia people use the plant as a remedy for severe post-delivery bleeding. The leaves are soaked in water, pressed and the filtrate drunk by these mothers. *Mondia* roots among its general curing properties are known to manage STDs (www.nationaudio.com).

2.4 THE RAT AS AN EXPERIMENTAL ANIMAL

Next to the mouse, the rat is the most extensively used experimental animal, particularly in the fields of nutrition, transplantation, immunology, genetics, cancer, pharmacology, physiology, neuroscience and aging research (Pass and Freeth, 1993). Rats, by a fortunate coincidence show a similarity to man in many of their physiological and medical processes. The Norway rat (*Rattus novergicus*), became the first mammalian species to be domesticated primarily for scientific purposes. The registration of medicinal products requires the provision of detailed data on safety, efficacy and pharmaceutical quality. Toxicity testing and reproduction studies provide such data and for the fertility and general reproduction studies, the rat is recommended in most countries (Gorrod, 1981).

The use of the lab rat has grown steadily. It is genetically well characterized and its larger size allows scientists to perform many procedures without any difficulty (Pass and

Freeth, 1993). It is economic and practical to use large numbers in an experiment to give greater statistical validity to results obtained because it breeds easily, profusely and continuously during its normal reproductive life span in the laboratory. Much is known about its physiology, anatomy genetics and behaviour (Waynforth and Brewer, 1980). Rats have a well-defined diurnal rhythm, being active during the dark and sleeping and resting during the light hours so feeding occurs at night and digestion during the early daylight hours. During this period, caged rats are easily handled, and also after repeated gentle handling they can be trained to receive some noxious stimuli such as injections with minimal restraint (Pass and Freeth, 1993),

Pass and others (1993) also reported that sexual maturity is reached by 8 weeks but some inbred strains take up to 12-16 weeks. Females are continuously polyoestrus, with a cycle of 4-6 days and will mate within 48 hours of parturition, with 50% fertility. Even though the males will attempt to mate with the females immediately, they accept the males only in early oestrus. Under good laboratory conditions, the life span of the rat is 2-4 years. The net organ weights per 100 g body weight for some organs are 0.04 g for both adrenal glands, 1.0 g for testes, 0.1 g for ovaries, 0.005 g for pituitary, 0.16 g for prostate glands and 0.3 g for seminal vesicles with semen removed (Waynforth and Brewer, 1980).

2.5. RECEPTORS OF THE REPRODUCTIVE SYSTEM

Drugs interact with specific elements of the reproductive system and this interaction results in some characteristic tissue response. These specific elements are the receptors.

Adrenoceptors are those that adrenominetic agents react with. Drugs that produce response by interacting with adrenoceptors are referred to as adrenoceptor agonists. Agents that inhibit responses mediated by adrenoceptor activation are known as adrenoceptor antagonists or adrenergic blocking agents (Westfall, 1990).

Hoffman (1992) reiterating on discoveries of Ahlquist (1948), proposed that α and β adrenoceptors exist. β_1-receptors have approximately equal affinity for adrenaline and noradrenaline whereas β_2 have a higher affinity for adrenaline than noradrenaline. α-receptors exhibit the potency series of adrenaline being greater or equal to noradrenaline and far greater than isoproterenol.

Stimulation of these receptors can cause a contraction or relaxation of effector organs. The uterus contains β-adrenergic receptors. A rat uterus preparation is very sensitive to adrenaline and moderately sensitive to noradrenaline (Livingston, 1970). β_2-receptors cause a relaxation of the vascular and uterine smooth muscle, and its effect is diminished by propranolol, which is a non-selective β-receptor blocking agent. Metaprolol, which is selective, is a β_1-receptor blocker (Westfall, 1990).

Studies have suggested that there is an existence of a non-adrenergic component prominent in the prostatic half of the vas deferens tube whiles there is the well known predominance of the adrenergic in the epididymal portion (Kitchen, 1984b). Electrical and mechanical responses of epididymal and prostatic regions of the vas deferens have been examined to investigate regional variation in purinergic and adrenergic mechanisms.

Noradrenaline was found to be significantly more potent in producing contraction in the epididymal segments than the prostatic segments whiles ATP was more potent in the prostatic segments than the epididymal segments (Sneddon and Machaly, 1992). The vas deferentia possess α_1 and α_2 adrenoceptors at post- and pre-synaptic sites respectively. α_1 receptors are those whose stimulation results in responses associated with the postsynaptic α -receptors of vascular smooth muscle whiles α_2-receptors are those whose stimulation results in responses associated with the presynaptic α -receptors of peripheral nerves (Westfall, 1990). The vas deferens contracts in response to adrenaline or noradrenaline and the receptors associated with this response are the α-adrenoceptors. Drugs with affinity for these receptors usually inhibit noradrenaline induced contraction and/ or induce similar contractions. Some α_1 antagonists decrease the maximum response of noradrenaline on vas deferens (Kitchen, 1984b). Studies show that this contractile effect of noradrenaline and adrenaline is solely through α_1- adrenergic receptors and that there are no α_2-adrenegic receptor-mediated contractile responses. This implies or suggests the vas deferens contains a homogenous population of α_1-adrenergic receptors mediating the contractile response to noradrenaline (Minneman *et al.*, 1983). The subtypes of α_1 and α_2-adrenoceptor mediating contractions of vas deferens contractions to nerve stimulation in vas deferens involve largely α_{1a}-adrenoceptors and purinoceptors. Contractions due to exogenous administration of noradrenaline involved both α_{1a} and α_{2a} adrenoceptors (Cleary *et al.*, 2003).

Responses to noradrenaline are unaffected by β-adrenoceptor antagonists. However, β_2-adrenoceptors do appear to be present in the mouse vas deferens since isoprenaline and

salbutamol inhibit the twitch response, an effect blocked by propranolol. So contraction caused by noradrenaline in the rat vas deferens could be due to β_2-adrenoceptors (Kitchen, 1984b) since in experiments performed, propranolol reduced effect of noradrenaline on the tissue.

The α_{1a}-adrenoceptor mediated contraction to noradrenaline of the rat prostatic vas deferens appears to consist of an initial phasic component due to release of intracellular Ca^{2+} from ryanodine-sensitive stores. These stores are depleted in the absence of extracellular Ca^{2+}. The α_1-adenoceptor mediating contraction of the rat epididymal vas deferens has been characterized in several studies as the α_1-subtype (quoting Burt *et al.*, 1995), but the subtype in the prostatic vas deferens has not been fully characterized (Burt *et al.*, 1998). Han and others (1987) reported that there are two subtypes of α_1-adrenoceptors which cause contractile responses through different molecular mechanisms. One subtype stimulates inositol phosphate (InsP) formation and causes contractions which are independent of extacellular Ca^{2+}, and the other does not stimulate InsP formation and causes contractions which require the influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels. These results suggested that neurotransmitters and hormones may control Ca^{2+} release from intracellular stores and influx through voltage-gated membrane channels through distinct receptor subtypes. In 1999, Honner and Docherty investigated the subtypes of α_1-adrenoceptors that mediate contractions of rat vas deferens. They reported that in the epididymal portions, isometric contraction is α_1-adrenoceptors mediated. Tonic contractions produced by exogenous agonists are mediated predominantly by α_{1A}-adrenoceptors, although a second subtype of

receptors may be additionally involved in phasic contractions. Contraction to nerve stimulation resembled the α_{1D}-adrenoceptors. Cleary *et al.*, 2004 confirmed that the contraction of the rat vas deferens to exogenous noradrenaline involved predominantly α_{1A}-adrenoceptors, but contractions to endogenous noradrenaline involved predominantly α_{1D}-adrenoceptors, particularly phasic contractions.

Vas deferens motility can be inhibited through inhibition of Ca^{2+} entry as well as by the effect of an adrenoceptor antagonist or just by the inhibition of the Ca^{2+}entry (Medina *et al.*, 2000). Receptor-mediated increases in intracellular Ca^{2+} levels can be caused by release from intracellular organelles and/or influx from the extracellular fluid. Noradrenaline released from sympathetic nerves acts on α_1-adrenoceptors to increase cystolic Ca^{2+} and promote smooth muscle contraction. But research results suggested that neurotransmitters and hormones may control Ca^{2+} release from intracellular stores and influx through voltage-gated membrane channels through distinct receptor subtypes, because α_1-adrenoceptors have recently shown to have different pharmacological properties in different tissues and therefore it has been proposed that different α_1-adrenoceptors subtypes may control mobilization of intracellular Ca^{2+} and gating of intracellular Ca^{2+} influx (Han *et al.*, 1987). Differences in contractile responses could also be caused by a reduction in the number of postjunctional α -adrenergic receptors or decreased receptor sensitivity (Turken *et al.*, 1999). Smooth muscle contraction nearly always involves a rise in intracellular Ca^{2+} or both. The α_{1a}-mediated contraction of the rat epididymal vas deferens seems to be dependant on the influx of extracellular Ca^{2+} through nifedipine-sensitive channels. Burt *et al.*, 1998 (quoting Burt *et al.*, 1996) stated that preliminary experiments have suggested that the whole contraction to noradrenaline

in the prostatic vas deferens was dependent on extracellular Ca^{2+}. *Vitis vinifera* hydroalcoholic leaf extracts reduced vas deferens contractions through the blockade of voltage dependent calcium channels with no β-adrenergic activity involved in this relaxatory effect (Gharib and Vakilzadeh, 2004).

The uterus is reported to be richly endowed with both α_1- and β-adrenergic receptors and catecholamines could alter uterine activity. Adrenaline caused dose-dependent reductions in uterine activity, blocked by propranolol, a β-adrenergic blocker (Segal *et al.*, 1998). Aqel and others (1991) (quoting the work of Van Breeman *et al.*, 1982) stated that contraction of the smooth muscle is also dependent on extracellular or intracellular source of Ca^{2+}. The spontaneous movement of smooth muscle is regulated by cycles of de- and re-polarization. *Andrographis paniculata* (Wess) is reported to induce relaxation of the uterus by blocking voltage operated calcium channels and inhibits Ca^{2+} influx. Uterine horns pretreated with oestradiol were incubated in Ca^{2+}-free De-Jalons solution and stimulated with KCl in order to produce depolarization of the membrane. The maximum contractile response induced by acetylcholine was moderately antagonized by *A. paniculata*. Results that were obtained strongly suggested that *A. paniculata* blockades voltage operated calcium channels inhibiting the entry of Ca^{2+} from the external medium (Burgos *et al.*, 2001). The relaxatory effect of *Vitis vinifera* leaf extracts on rat uterus has also been reported and postulated that the extract relaxed through the blockade of voltage dependent calcium channels (Gharib and Vakilzadeh, 2004).

The uterus is innervated by sympathetic nerves and possibly a parasympathetic supply which runs to the wall of the tissue. In the rat uterus, β-receptors remain constant, but α -receptors appear under the action of estrogen. The β-adrenergic receptor responses predominate but it is sometimes preceeded by a transient α -adrenergic receptor mediated contraction for mixed α - and β- agonists. Since the isolated uterus has no inherent tone, relaxation can only be observed well by physiological antagonism to the contractile responses caused by drugs such as acetylcholine β-receptor-mediated antagonists such as propranolol (Kitchen, 1984a).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. EXPERIMENTAL ANIMALS

Albino rats were used for the experiments. Their weights ranged between 30 g-300 g depending on the type of experiment being performed. The animals were obtained, bred and kept in the animal house in the Faculty of Pharmacy under normal environmental conditions, i.e. ambient temperature (25-30°C) and relative humidity range, 65-86%. Rats were fed with a standard chew diet (made of maize, vitamins, fishmeal, wheat brand produced by GAFCO, Tema-Ghana) and water *ad libitum*.

3.2 SOURCE OF PLANT MATERIALS

Dried roots of *M. whitei* were obtained from the Centre for Research into Plant medicine, Akwapim Mampong. Alcoholic extracts were then produced from these roots.

3.3 GENERAL METHODS

3.3.1 EXTRACTION

Dried roots were pulverized in a hammer mill into powder to enhance extraction. Using the Soxhlet apparatus, an alcoholic extract was obtained from the powdered roots (1.5 kg) with 70% ethanol (5 litres) at 60°C for three days with occasional stirring. Filtration was done after the 3rd day. Using a vacuum rotary evaporator, the alcoholic filtrate was concentrated at a low temperature, under reduced pressure and dried further in a water bath at 60°C (Choudhary *et al.*, 1991). The dried extract (200 g) was kept in a dessicator.

Stock solutions were freshly prepared and depending on the experiment, different concentrations were prepared by serial dilutions for administration.

Equivalent human doses by weight of the *Mondia whitei* extract that were used on the rats mostly throughout the experiments were based on what would be required by the average 70 kg man. These Doses were half the normal dose (2.05 mg/kg), the normal dose (4.11 mg/kg) and twice the normal dose (8.22 mg/kg).

3.3.2 RANDOMIZATION AND IDENTIFICATION OF ANIMALS

For each of the experiments, the animals were randomly divided into groups, each containing at least two rats; the number used is dependent on the type of experiment to be performed. These experiments were 1/. Toxicity experiments 2/. *In vivo* experiments 3/. *In vitro* experiments

3.4 TOXICITY TESTS

In Keeping with WHO norms and recommendations, general toxicity studies in rats were undertaken using a 1967 WHO protocol (Al-Bekairi *et al.*, 1991). In accordance with this protocol teratogenicity activity and related studies should be performed at least at three different concentrations of the drug in use, (1) with acute toxic effects on the animals; (2) inducing chronic toxicity and (3) an intermediate dose between the two concentrations as sub-acute. Rats in each of the 3 groups were observed for changes in bodyweight, faeces output and general health over the period. At the end of the

stipulated period, these rats were sacrificed and histological profiles of the liver and kidneys studied (Medeiros *et al.*, 2000).

3.4.1 EXTRAPOLATION OF RAT DOSES FROM HUMAN

The single dosage for an average 70 kg man is chewing about 6 pieces of cylindrical *Mondia* root pieces each cut with dimensions of about 3 cm length by 1 cm diameter. A crude extrapolation from this showed that after the extraction, each set of six root pieces is about 0.288 g of the crude extract *M. whitei*. From this rough estimation equivalent doses based on weight were calculated for each rat.

3.4.2 ACUTE TOXICITY

Healthy adult rats of mixed sexes with weights between 130 g-250 g were put into five groups with two animals in each group. Two sets of experiments were done. The five equivalent man dose levels for the rats in the first experiment were 2.05 mg/kg (1/2x normal dose), 4.11 mg/kg (normal dose), 8.22 mg/kg (2x normal dose), 16.44 mg/kg (4x normal dose), 32.88 mg/kg (8x normal dose). Equivalent man dose levels administered to rats of the 5 groups in the second experiment were 64.76 mg/kg (16x normal dose), 131.52 mg/kg (32x normal dose), 263.04 mg/kg (64x normal dose), 526.08 mg/kg (128x normal dose), or 1052.16 mg/kg (256x normal dose) of the extract. The animals were observed closely for three days after drug administration for toxic symptoms. Administration was carried out once, orally by gastric intubation.

3.4.3 SUB-ACUTE TOXICITY

Healthy albino rats of mixed sexes weighing between 125-160 g were put into four groups, each made of five rats. The different groups were either given distilled water or treated with 2.05 mg/kg (half the normal dose), 4.11 mg/kg (the normal), or 8.22 mg/kg (twice the normal dose) of the *Mondia* root extract. Each dose was administered 3 times daily for one month. At the end of the administration period, animals from each group were sacrificed and histological profiles of the liver and kidney studied.

3.4.4 CHRONIC TOXICITY

Healthy albino rats with weights 20-80 g were put into four groups consisting of five rats in each group. Rats in different groups were either treated with 2.05, 4.11, 8.22, mg/kg of the *Mondia* extract or were given distilled water (control group). The dosing was three times daily for three months. At the end of the administration period, animals from each of the groups were sacrificed and histological profiles of the liver and kidneys studied.

3.4.5 HISTOLOGICAL PROFILE STUDIES

Kidneys were fixed in Bouins fluid, embedded in paraffin, sectioned and stained with haemotoxylin and eosin for the histological observations to be done. The numbers of Malpighian corpuscles were easily counted by identifying the juxtaglomerular apparatus. Histometric measurements using ocular and stage micrometers such as the diameters and numbers of the glomeruli were done by a random selection of about 10 sections in each histological section of the kidney organs from each of the rats in the experimental groups.

Similar numbers of sections of the liver from each rat from all the experimental groups were also studied and the states of the hepatocytes were studied.

3.5 *IN VIVO* EXPERIMENTS

3.5.1 EVALUATION OF ANDROGENIC OR ANTI-ANDROGENIC ACTIVITY OF *M.WHITEI*

Colony bred immature male albino rats; four to eight weeks old and weighing between 45-120 g were used for evaluation of androgenic or anti-androgenic activity. Pass and Freeth, 1993 indicated that puberty usually sets in for rats between the weights of 150-200 g and sexual maturity is reached by eight weeks but for some inbred strains maturity will take up to twelve to sixteen weeks. They were divided into four groups, two of the four groups consisted of nine rats each and the other two consisted of twenty-seven rats each. The first group consisting of nine rats served as (positive) controls and received distilled water only. The second group also consisting of nine rats served as a negative control and received testosterone at 1 mg/kg body weight applied subcutaneously. A stock solution of 25mg/ml testosterone diluted in a 1000 ml of arachnid oil was used. The third group of twenty-seven rats were grouped into 3 batches of nine rats each were given three level doses of the ethanolic extract of *M. whitei* at 2.05 mg/kg (half the normal dose), 4.11 mg/kg (the normal dose), or 8.22 mg/kg (twice the normal dose) mg/kg bodyweight applied orally. The fourth group received in addition to testosterone (1 mg/kg bodyweight), test doses of the extract at 2.05, 4.11, or 8.22 mg/kg bodyweight. A group of nine rats from the fourth group were given each of the three extract dose

levels and testosterone combination. All the above experiments lasted for three weeks and administration for each set up was given once daily.

Body weights were taken at the beginning of experiments. Body weights were again monitored weekly of the rats before being sacrificed at the end of the each experimental period. Percentage body weights were then calculated and average body weights obtained for each group. The testes, epididymis, vas deferens, ventral prostate and seminal vesicles were dissected out, freed from surrounding tissues and weighed quickly on a sensitive metler scale balance. Percentage organ weights were calculated with the weights of organs obtained and body weights just before sacrifice, for each rat. The testes, seminal vesicles and ventral prostate were fixed in Bouins fluid, embedded in paraffin, sectioned at 6um and stained with haematoxylin and eosin for histological observation. Histometric measurements such as diameters of the epithelial glandular lining of the seminal vesicles and ventral prostates were made by random selection of about 10 circular sections from each histological section of these organs by using ocular and stage micrometers. Average widths were calculated for each dose level of *M. whitei*, testosterone and controls. Some studying and observations of the freed testes from rats at the end of each week of treatment were done by random selection of 30 circular sections. The nature of different spermatogenic elements, tracing spermatogenesis in each couple of testes from each rat from all the experimental groups were observed (Hiremath *et al.*, 1997).

Statistical analysis was carried out to ascertain whether, percentage body and organ weights compared at different dose levels in the various experiments had either been time or dose dependent or both.

3.6 *IN VITRO* EXPERIMENTS

3.6.1 EFFECT OF *M. WHITEI* ON ISOLATED RAT VAS DEFERENS.

A male albino rat was injected 24 hours prior to the experiment with 3 mg/kg of reserpine per body weight. Reserpine is reported to deplete noradrenaline from tissues such as the brain, heart, vas deferens and uterus (Satia and Goyal, 1993). Super sensitivity was induced through pretreatment with reserpine (Hershman *et al.*, 1993). The rat was killed with a sharp blow to the head, the abdomen was then opened and the vas deferens freed from all other tissues and fat. The tube-like structure was tied to the tissue suspender in a 10ml organ bath (Harvard apparatus Ltd, U.K) containing Krebs Physiological Solution aerated and maintained at 32°C. The preparation was allowed to acclimatize with its new environment for at least 30 minutes. With a kymograph speed of 2 mm/min, a time cycle of 7-10 minutes and a drug contact time of about 1-2 minutes, cumulative dose-responses were obtained for noradrenaline and then secondly for the effect of *M. whitei* alone. In a third experiment, graded dose responses were obtained for noradrenaline in the presence of 0.33×10^{-7} M and 0.5×10^{-7} M propranolol. In the last experiment for the vas deferens tissue, graded dose responses were obtained for noradrenaline in the presence of 100 mg/ml *M. whitei* to determine if *M. whitei* antagonizes the effect of noradenaline. The contractile effect of noradrenaline was measured and effect of *M. whitei* on the contractile effect of noradrenaline on the tissue observed. The experiments were repeated and

isotonic contractions were recorded again with the kymograph. The mean responses were converted into percentage maximum responses. Log dose-response curves were plotted.

3.6.2 EFFECT OF *M.WHITEI* ON ISOLATED RAT UTERUS

A non-pregnant female rat was injected intramuscularly 24 hours prior to the experiment with 0.1 mg/kg of Stilboestrol, in order to get consistent responses during the experiment. Stimulation of uterus can be due to the occurance of oestrus symptoms accompanied by follicular growth and progesterone content in the blood. Administration of stilboestrol reduces the possible rise of progesterone in uterine biopsies as well as in the blood (Nitchelm and Vander, 1976). The rat was killed with a sharp blow to the head, dislocating the neck, the abdomen opened and the uterine horns freed from fat and all other tissues. These are often easily distinguished by their pink colouration. These were then transferred to a petri dish containing De-Jalons Ringer solution. Depending on the size of the uterus, each tube was cut longitudinally resulting in a sheet of muscle, which was further sliced into narrower longitudinal strips and used as the preparation. The sheets of muscles were then tied at both the top and bottom with the bottom attached to the tissue suspender and the bottom to the writing lever with a thread. The preparation was then transferred to a 10 ml organ bath (Harvard apparatus Ltd, UK) containing De-Jalons physiological solution, aerated with O_2 (95%) and CO_2 (5%) and maintained at 32°C. The preparation was allowed to acclamatize with its new environment for at least 30 minutes. With a time cycle of 3 minutes, contact time of 14-30 seconds and a kymograph speed of 4 mm/min, cumulative dose responses were obtained for adrenaline

and *M. whitei*. Graded dose responses were also obtained for adrenaline and *M. whitei* using $0.33x10^{-7}$ M, $0.5x10^{-7}$M and $1.0x10^{-7}$M Propranolized De-Jalons (i.e. in the presence of $0.33x10^{-7}$ M, $0.5x10^{-7}$ and $1.0x10^{-7}$M Propronolol).

The relaxation and contractile effects were measured. The experiments were then repeated and the mean responses were converted into percentage maximum responses. Log Dose- Response curves were then obtained by iterative curve fitting on a computer.

3.6.3 PHOTOMICROGRAPHS

Photomicrographs of sections or slide prepared were taken under high and low magnifications. Magnification powers were indicated for photomicrographs that were for only general examination and not measurements whiles measurement bars were used to indicate differences and magnification for sections that sizes, widths and other measurements were needed.

Sections involved in toxicity tests for general histological examination (Plate 1E) as well as numbers of renal corpuscles (Plate 2C) had magnifications indicated by magnification powers and those for measurements such as glomerular sizes, measurement bars were used (Pate 1B). Photomicrographs of sections for *in vivo* experiments that were for only general examination such as only tracing spermatogenesis, had magnifications illustrated by magnification powers (Plate 5A) and measurement bars used for sections in which measurements such as widths of epithelial lining were done (Plate 9F).

CHAPTER 4

4.0. RESULTS

4.1. TOXICITY TESTS USING *MONDIA WHITEI*

4.1.1. ACUTE TOXICITY

The administration of doses 2.05 mg/kg (half the normal dose), 4.11 mg/kg (normal dose), 8.22 mg/kg (2 x normal dose), 16.44 mg/kg (4 x normal dose), 32.88 mg/kg (8 x normal dose) of the *Mondia whitei* extract produced no acute toxic effects for a maximum of 3 days. Even with higher doses 64.76 (16 x normal dose), 131.52 (32 x normal dose), 263.04 (64 x normal dose), 526.08 (128 x normal dose), or 1052.16 (256 x normal dose) mg/kg for a day, the animals looked physically strong and active with no visible autonomic and/or behavioral changes up to 3 days post treatment. There were no signs of illness even after 7 days of administration.

4.1.2. SUB-ACUTE TOXICITY

The intake of experimental ration containing half the normal dose (2.05 mg/kg), the normal (4.11 mg/kg) or twice the normal doses (8.22 mg/kg) of *M .whitei* over a month was tolerated. No signs of sickness or abnormal behaviours were observed, animals were all healthy, and active. They looked physically strong with no visible toxic effects. However, all rats that were administered with twice the normal dose passed out softer faecal pellets than those that were put on lower doses.

A careful study of the microscopic slides for which the photomicrographs (Plate 1A-E) of kidney sections were developed for the untreated and *M. whitei*-treated rats, showed there were no significant changes in the histological profiles of this organ. The magnification details revealed that the cortical parenchyma, the renal corpuscles and glomeruli for both the *M. whitei*-treated (at all dose levels) and controls groups were all intact with no noticeable destruction. Bowman's capsules were normal with no disorganization. The entire cellular mass was not fused together or damaged but well defined (Plates 1A-C, 1E) except for one rat treated with 8.22 mg/kg *M. whitei* whose kidney section showed no defined Bowman's space or cavity but the entire cellular mass well developed (Plate 1D). However, one slide preparation (½ x normal dose) showed some areas or sections with cortical cells being swollen, fluid packed and compacted glomeruli. The Bowman's cavity between the outer layer of the glomerulus and the inner layer of the Bowmans capsule is not well defined showing a fused glomerulus with the Bowmans capsule (Plate 1F). Another rat treated with an extreme dose showed a few calcified deposits in the histological section (Plate 2A).

Administration of *M. whitei* at mean dosage of 2.05 mg/kg per body weight after one month of dosing resulted in quite a significant decrease $P<0.05$ in the relative numbers of renal corpuscles when compared to the controls (Plate 2C). However, the ethanolic extract at a dose level of 8.22 mg/kg (2xnormal dose) for that same one month significantly increased the renal corpuscle numbers when compared to a dose level of 2.05 mg/kg, $P<0.01$ (Plate 2D), but compared to the controls, no significant changes occurred, $P<0.05$, Plate 2B (Table 1).

Table 2 indicates that measurement of the relative sizes of the renal corpuscles showed that the level of 4.11 mg/kg, the normal dose (Plate 1C) caused quite significant increases when compared to the controls (Plate 1A) and dose level of 2.05 mg/kg (Plate 1B). But administration of the extract at a dose level of 8.22 mg/kg reduced sizes significantly compared to dose level of 4.11 mg/kg. Histological evidence of these effects is shown in Plate 1D).

After studying the microscopic slides from which some the photomicrographs of liver sections were developed, for the controls rats(Plate 2E) on one hand, rats treated with 2.05 mg/kg, 4.11 mg/kg, 8.22 mg/kg (Plate 2F) of the *M. whitei* extract on the other hand, there were no observable changes in the histological profile of this organ. The magnification details reveal intact hepatocytes, still polyhedral with large nuclei, tightly packed with sinusoid lining cells between them

Table 1 The effect of *M. whitei* on glomerular numbers within kidneys of rats during sub acute toxicity tests. All values are mean ± SEM. * Significantly different from control $p< 0.05$, @@ Significantly different from ½ x Normal dose $p< 0.01$.All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Tests

Dosage (mg/kg body weight)	Mean ± SEM (Numbers)
0(Control)	2.667 ± 0.182
2.05 (½ x Normal)	1.967 ± 0.195*
4.11 (Normal)	2.300 ± 0.231^{ns}
8.22 (2 x Normal)	2.900 ± 0.1907@@

Table 2 The effect of *M. whitei* on glomerular sizes within kidneys of rats during sub acute toxicity tests. All values are mean ± SEM. *Significantly different from Control $p<0.05$, @Significantly different from ½ Normal dose $p<0.05$, ˙ Significantly different from Normal dose $p<0.05$. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Tests

Dosage (mg/kg body weight)	Mean ± SEM (Sizes)
0(Control)	0.071 ± 0.002
2.05 (½ x Normal)	0.077 ± 0.002^{ns}
4.11 (Normal)	0.133 ± 0.028* @
8.22 (2 x Normal)	0.075 ± 0.001˙

PLATE 1 KIDNEYS OF RATS TREATED SUB-ACUTELY (1 MONTH) WITH *M. WHITEI* SHOWING RENAL CORPUSCLES. (X400)

A. Transverse section through the kidney of a control rat illustrating Bowmans capsule. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is very defined and the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

B. Transverse section through the kidney of a 2.05 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width was not different from that of the control rat. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is very defined and the glomerulus (G) well formed. Cellular organization is normal with proximal and distal convoluted tubules seen.

C. Transverse section through the kidney of a 4.11 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width is shown to be relatively larger than the other treatments and the controls. Magnification details show the visceral and parietal layers. The Bowman's cavity or space (BC) is very defined and the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

D. Transverse section through the kidney of a 8.22 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width is shown to be relatively smaller than the 4.11 mg/kg *M. whitei*-treated rat. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is not very defined but the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

E. Transverse section through the kidney of an 8.22 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space is well defined and the glomerulus (G) well formed. Cellular organization is normal with proximal and distal convoluted tubules seen.

F. Transverse section through the kidney of a 2.05 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. Magnification details show fluid filled cortex. The Bowman's cavity (BC) or space is not defined.

Plate 1 KIDNEYS OF RATS TREATED SUB-ACUTELY (1 MONTH) WITH *M. WHITEI* SHOWING RENAL CORPUSCLES. (X400)

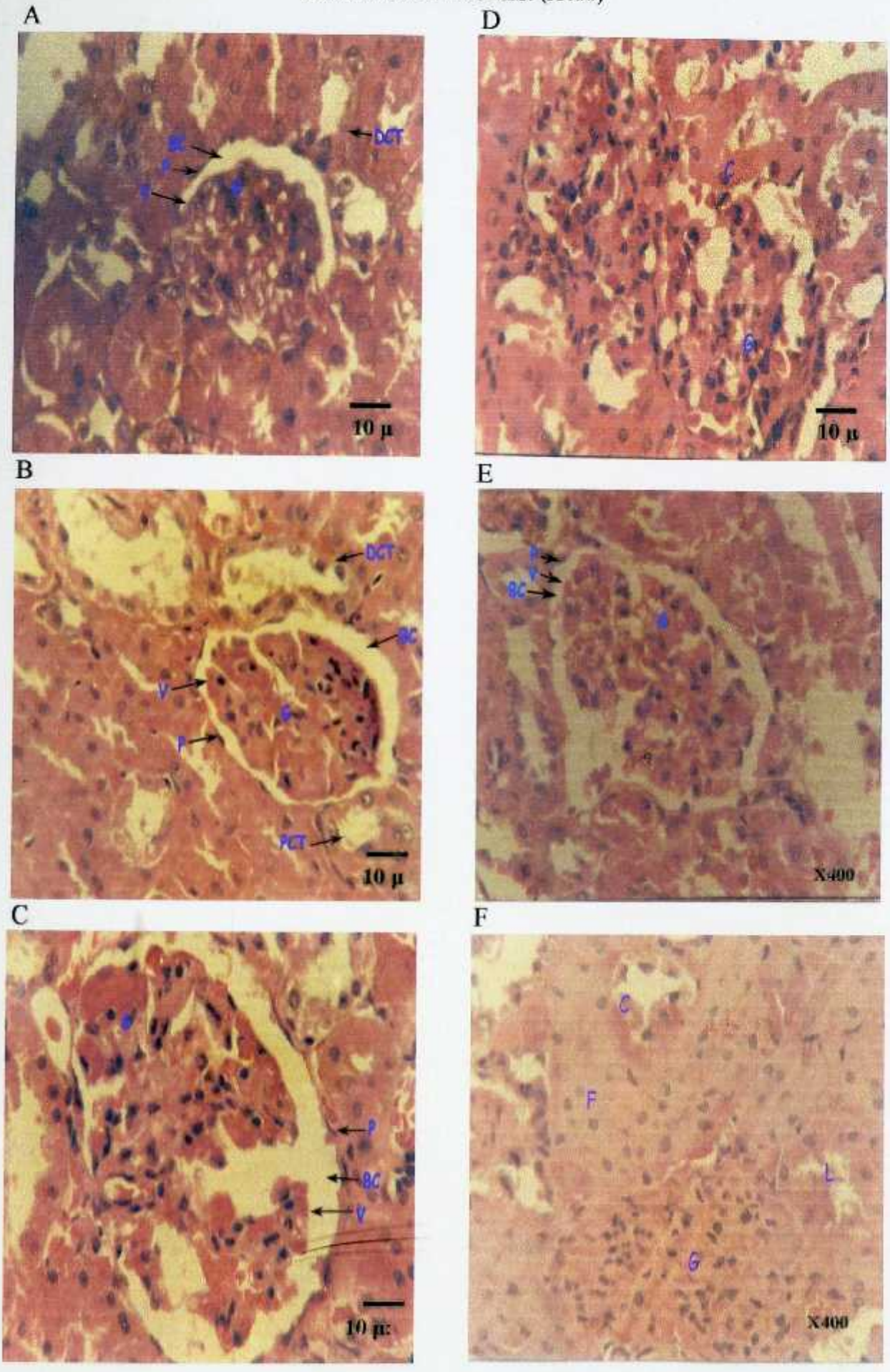


PLATE 2 KIDNEYS AND LIVER OF RATS TREATED SUB-ACUTELY (1 MONTH) WITH *M. WHITEI* SHOWING CELLULAR ORGANISATION.

A. Transverse section illustrating the kidney of a control rat showing calcified deposits within the lumens of the cortical cells. (X400)

B. A lower magnification illustrating a transverse section of a control rat showing a normal cellular organisation with renal corpuscles showing glomerulus (G) and Bowman's space (BC). (X100)

C. Transverse section of the kidney of a 2.05 mg/kg *M. whitei*-treated rat at a lower magnification. A normal cellular organization with renal corpuscles showing glomerulus (G) and Bowman's space (BC). The number of renal corpuscles seen to be less. (X100)

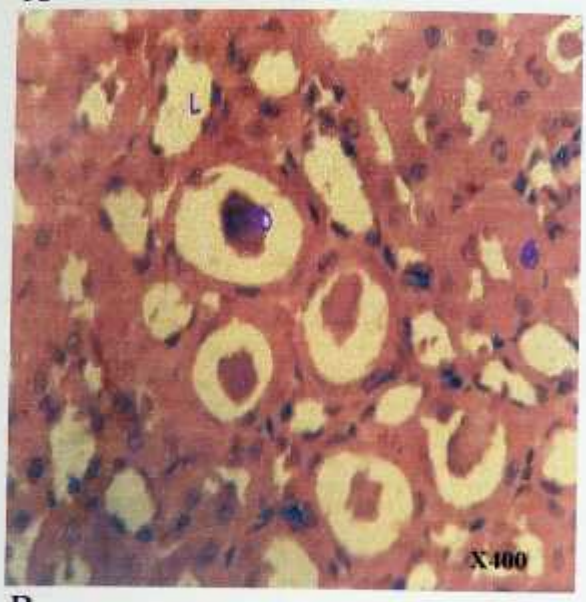
D. Transverse section of the kidney of an 8.22 mg/kg *M. whitei*-treated rat at a lower magnification. A normal cellular organization with renal corpuscles showing glomerulus (G) and Bowman's space (BC). The number of renal corpuscles seen to be more. (X100)

E. Low magnification of liver of a control rat showing liver hepatocytes (H) and hepatic venule (L). Hepatic cortical cells are polyhedral, largely nucleated and well defined. (X100)

F. Transverse section of the liver of an 8.22 mg/kg *M. whitei*-treated rat at a lower magnification illustrates normal cellular organization with polyhedral hepatic cortical cells (H) and prominent venules (L). (X100)

Plate 2 KIDNEYS AND LIVER OF RATS TREATED SUB-ACUTELY (1 MONTH) WITH *M. WHITEI* SHOWING CELLULAR ORGANISATION.

A



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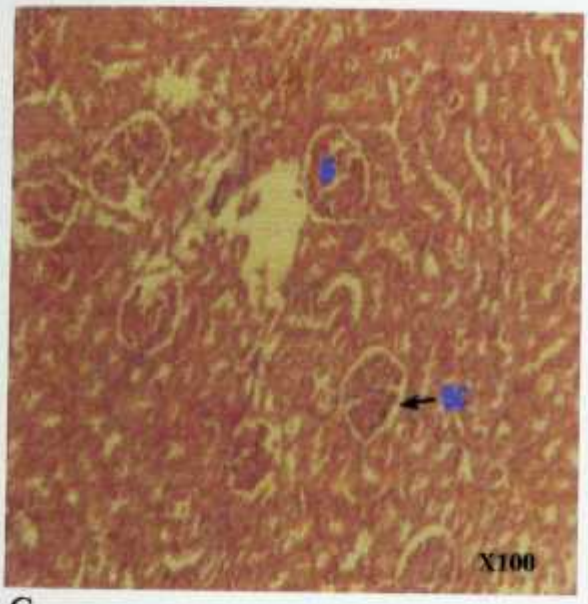

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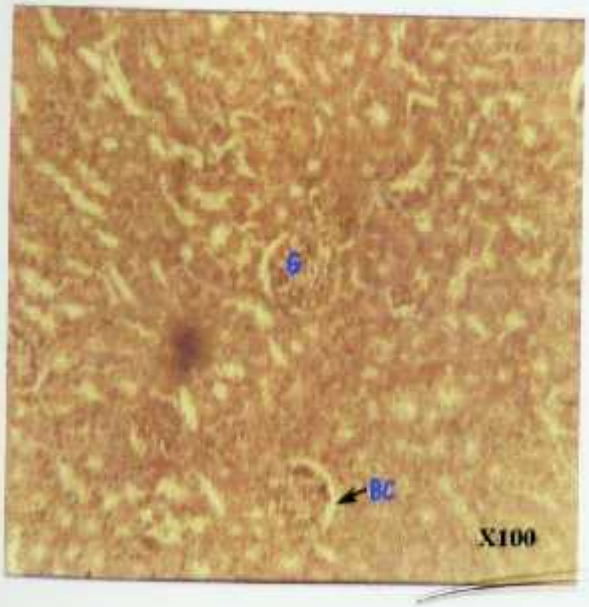

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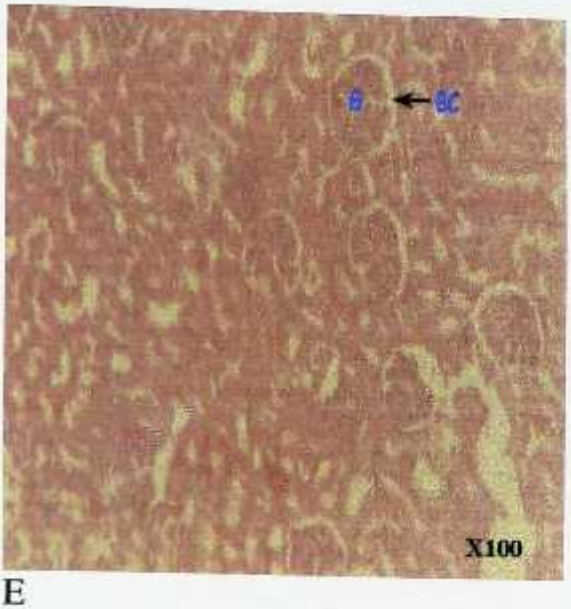

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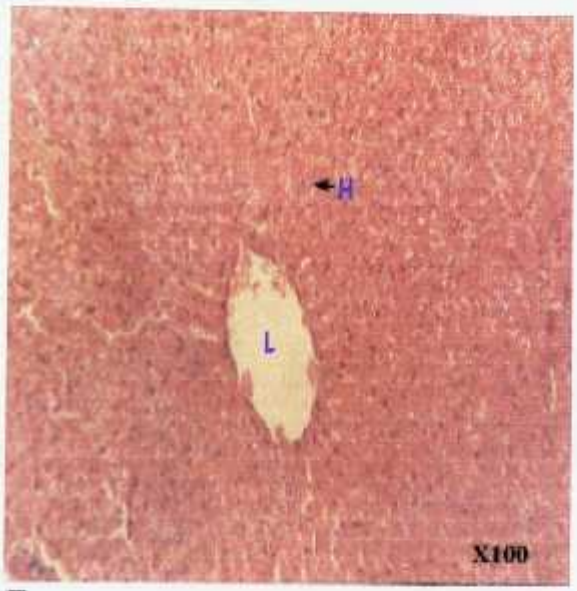
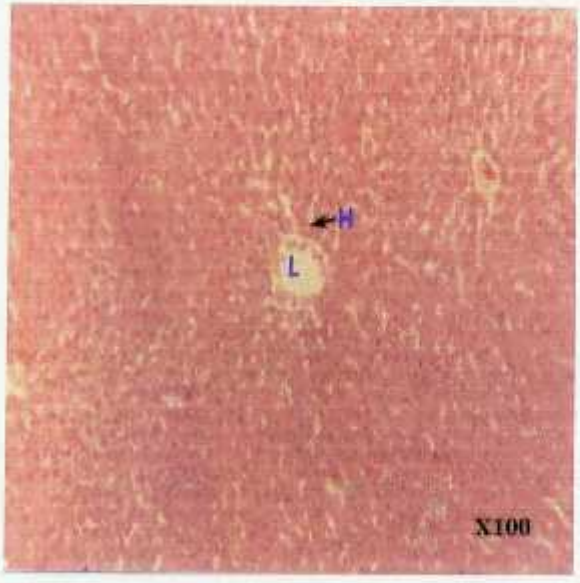


F



4.1.3. CHRONIC TOXICITY

Generally, animals that were treated with 2.05 mg/kg, 4.11 mg/kg, 8.22 mg/kg and 16.44 mg/kg of the *M. whitei* extract were physically very healthy. Two deaths were recorded. In the first the rat displayed general deterioration, and died after 2 days. The second death occurred within a longer period during the fifth week of treatment; the rat exhibited circular movements due to a neck tilt and apparently could not be as active both in movements and feeding as the physically normal ones. Three rats at a point in time had their hair bitten off close to skin by other rats in the same cage.

After studying the microscopic slides and the photomicrographs (Plates 3A-3F) of kidney sections, for the controls(Plates 3A) and rats treated with 2.05 mg/kg(Plate 3B), 4.11 mg/kg (Plate 3C), 8.22 mg/kg (Plate 3D) and 16.44 mg/kg (Plate 3E) of the *M. whitei* extract, there were no observable changes in the histological profile of this organ except for one of the kidney sections of rats treated with 4.11 mg/kg of the *M. whitei* extract that showed some areas with cortical cells being swollen, fluid packed and some pink colorations. The Bowman's space or cavity between the visceral and parietal layers of the Bowman's capsule was not well defined. The glomerulus looks compacted with the Bowmans capsule (Plate 3F).

After three months of dosing, no significant changes in the relative numbers and sizes of renal corpuscles both for the 3 dose levels of extract and the control rats (Table 3 and Table 4 respectively) were observed.

Table 3 The effect of *M. whitei* on glomerular numbers within kidneys of rats during chronic toxicity tests. All values are mean ± SEM (ns $p>0.05$) Not significantly different from controls and other doses. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Tests.

Dose (mg/kg bodyweight)	Mean ± SEM (Numbers)
0 (Control)	2.767 ± 0.149
2.05 (*M. whitei*)	3.450 ± 0.223^{ns}
4.11 (*M. whitei*)	2.950 ± 0.185^{ns}
8.22 (*M. whitei*)	3.067 ± 0.151^{ns}
16.44(*M. whitei*)	2.967 ± 0.139^{ns}

Table 4 The effect of *M.whitei* on glomerular sizes within kidneys of rats during chronic toxicity tests. All values are mean ± SEM. (ns $p>0.05$) Not significantly different from controls and other doses. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Tests.

Dose (mg/kg bodyweight)	Mean ± SEM (Sizes)
0 (Control)	0.071 ± 0.002
2.05 (*M. whitei*)	0.100 ± 0.028^{ns}
4.11 (*M. whitei*)	0.074 ± 0.002^{ns}
8.22 (*M. whitei*)	0.073 ± 0.001^{ns}
16.44(*M. whitei*)	0.075 ± 0.002^{ns}

A careful study of the microscopic slides and the photomicrographs (Plates 4A-4F) of liver sections, for the controls (Plate 4A) and *M. whitei*-treated rats (Plate 4A), showed that there were no observable changes in the histological profile of this organ. The magnification details reveal intact hepatocytes, polyhedral in shape with large nuclei, still tightly packed with sinusoidal lining cells between them. Both the treated and non-treatment groups were all intact with no noticeable destruction.

PLATE 3 KIDNEYS OF RATS TREATED CHRONICALLY (3 MONTHS) WITH *M. WHITEI* SHOWING RENAL CORPUSCLES. (X400)

A. Transverse section through the kidney of a control rat illustrating Bowman's capsule. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is well defined and the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

B. Transverse section through the kidney of a 2.05 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width was not different from that of the control rat. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is well defined and the glomerulus (G) well formed. Cellular organization is normal with proximal and distal convoluted tubules seen.

C. Transverse section through the kidney of a 4.11 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width is shown to be the same as the other treatments and the controls. Magnification details show the visceral and parietal layers. The Bowmans cavity or space (BC) is very defined and the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

D. Transverse section through the kidney of a 8.22 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. The capsule size or width is shown to be the same as the other treatments and the controls. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space (BC) is very defined and the glomerulus (G) well formed. Cellular organization is normal with proximal (PCT) and distal convoluted tubules (DCT) seen.

E. Transverse section through the kidney of a 16.44 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. Magnification details show the visceral (V) and parietal layers (P). The Bowman's cavity or space is well defined and the glomerulus (G) well formed. Cellular organization is normal with proximal and distal convoluted tubules seen.

F. Transverse section through the kidney of a 4.11 mg/kg *M. whitei*-treated rat illustrating Bowman's capsule. Magnification details show fluid filled cortex. (BC) or space is not defined.

Plate 3 KIDNEYS OF RATS TREATED CHRONICALLY (3 MONTHS) WITH *M. WHITEI* SHOWING RENAL CORPUSCLES. (X400)

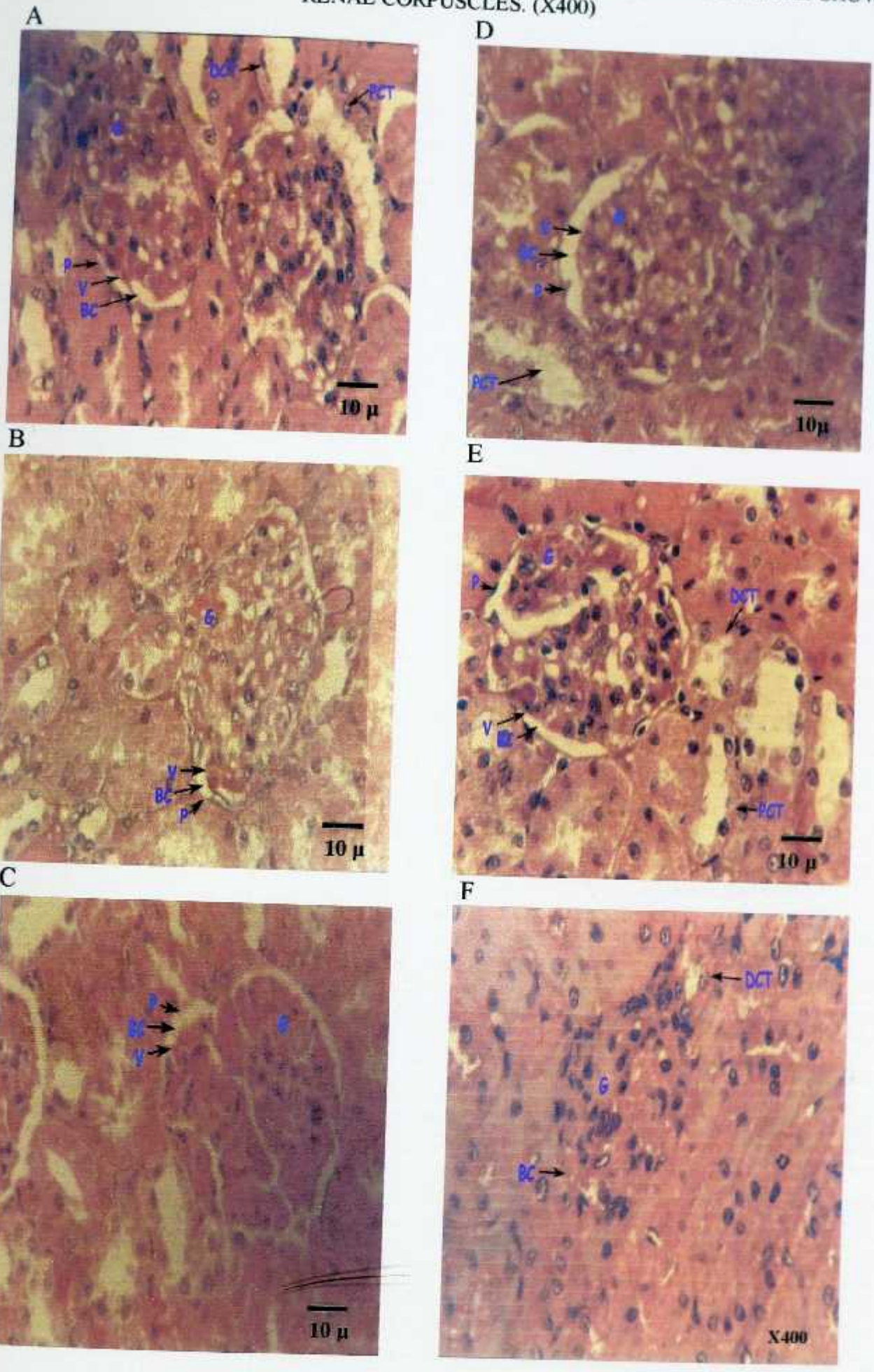


PLATE 4 KIDNEYS AND LIVER OF RATS TREATED CHRONICALLY (3 MONTHS) WITH *M. WHITEI* SHOWING CELLULAR ORGANISATION.

A. High magnification of liver of a control rat showing liver hepatocytes (H) and hepatic venule (L). Hepatic cortical cells are polyhedral, largely nucleated and well defined. (X400)

B. Transverse section of the liver of a 16.44 mg/kg *M. whitei*-treated rat at a lower magnification illustrates normal cellular organization with polyhedral hepatic cortical cells (H) and prominent venules (L). (X400)

C. Low magnification of liver of a control rat showing liver hepatocytes (H) and hepatic venule (L). Hepatic cortical cells are polyhedral, largely nucleated and well defined. (X100)

D. Transverse section of the liver of a 4.11 mg/kg *M. whitei*-treated rat at a lower magnification illustrates normal cellular organization with polyhedral hepatic cortical cells (H) and prominent venules (L). (X100)

E. Transverse section of the liver of an 8.22 mg/kg *M. whitei*-treated rat at a lower magnification illustrates normal cellular organization with polyhedral hepatic cortical cells (H) and prominent venules (L). (X100)

F. Low magnification of liver of a 16.44 mg/kg *M. whitei*-treated rat showing liver hepatocytes (H) and hepatic venule (L). Hepatic cortical cells are polyhedral, largely nucleated and well defined. (X100)

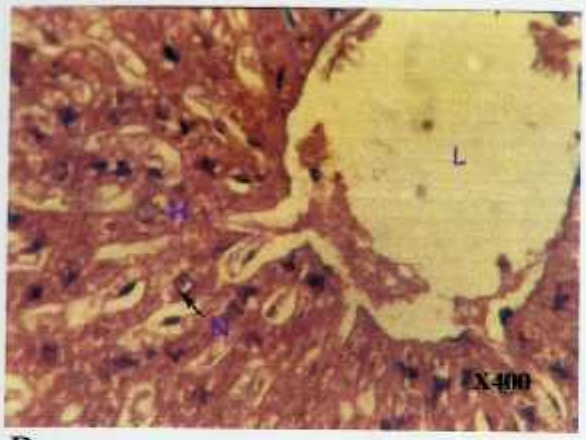
F. Transverse section of the liver of an 8.22 mg/kg *M. whitei*-treated rat at a lower magnification illustrates normal cellular organization with polyhedral hepatic cortical cells (H) and prominent venules (L). (X100)

G. A lower magnification illustrating a transverse section of the kidney of a control rat showing a normal cellular organisation with renal corpuscles showing glomerulus (G) and Bowmans space (BC). (X100)

H. Transverse section of the kidney of an 8.22 mg/kg *M. whitei*-treated rat at a lower magnification. A normal cellular organization with renal corpuscles showing glomerulus (G) and Bowman's space (BC). The number of renal corpuscles are seen to be equal. (X100)

Plate 4 KIDNEYS AND LIVER OF RATS TREATED CHRONICALLY (3 MONTHS) WITH *M. WHITEI* SHOWING CELLULAR ORGANISATION

A



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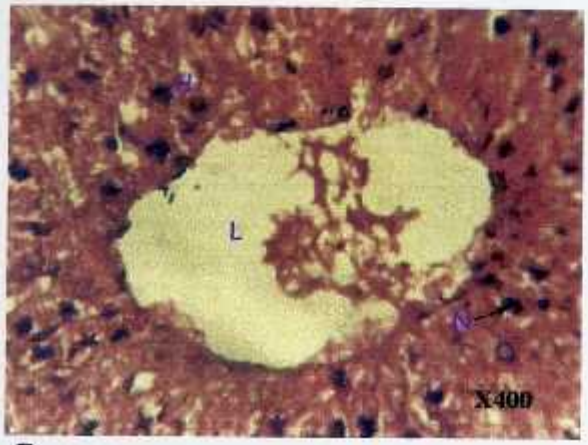

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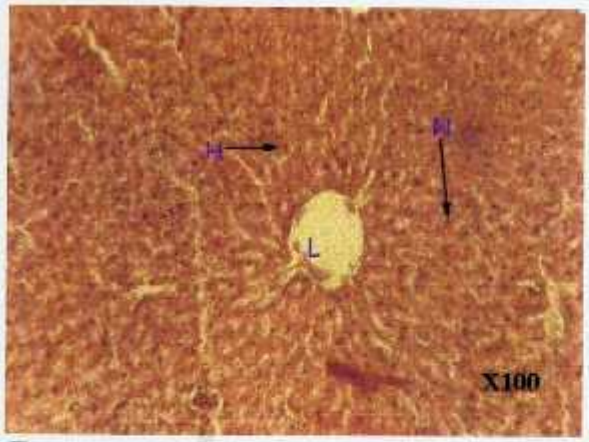

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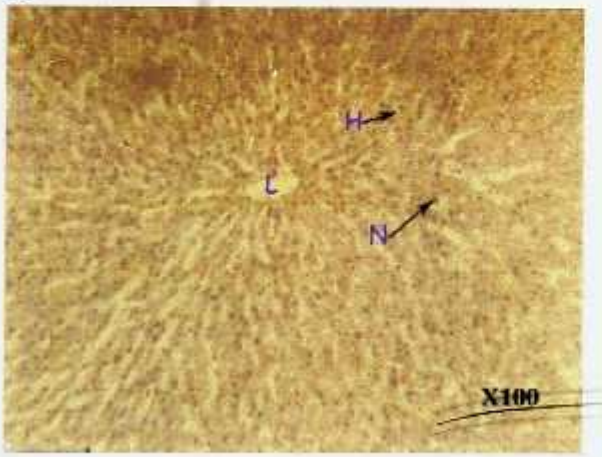

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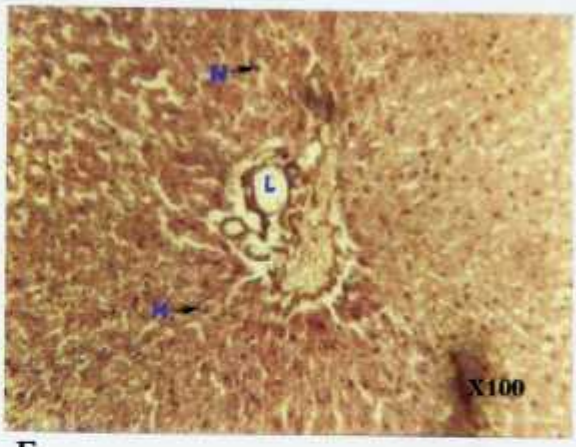

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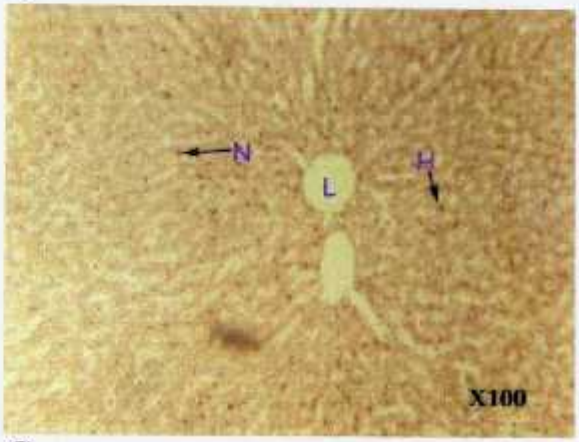

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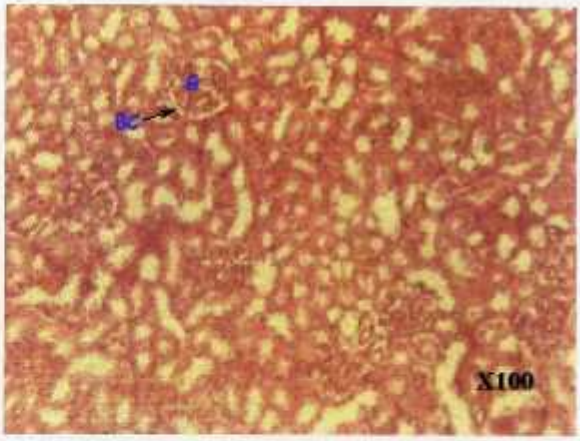
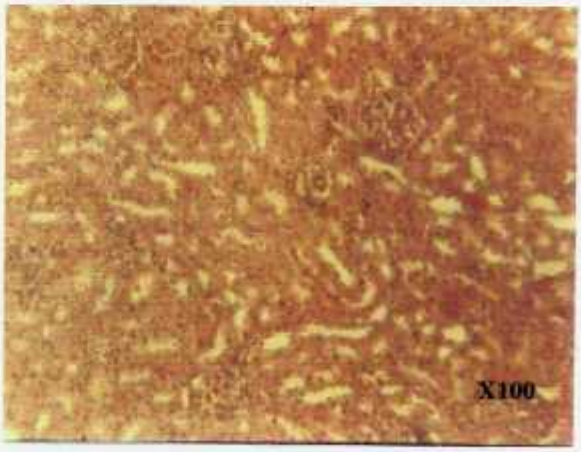


H



4.2 *IN VIVO* EXPERIMENTS

4.2.1. EFFECT ON BODYWEIGHT.

With the colony bred immature rats that were used in the experiments were divided into four groups and given different doses of the ethanolic extract of *M. whitei* and testosterone. Body weights were tracked weekly for the period of the experiment. A natural body weight increase was observed by the end of the 3 week experimental period for all the animals. Significant changes in body weights were observed during the first and second weeks of treatment. Both *M. whitei* and the testosterone-treated rats showed very significant increases in body weight, during the first week of treatment with respect to the controls. Compared to the controls, *M. whitei*-treated rats at a dose of 2.05 mg/kg ($p<0.01$), 4.11 mg/kg ($p<0.001$) and 8.22 mg/kg ($p<0.001$) and the testosterone treated rats showed very significant increases in body weight, during the second week of treatment. The combined treatments with *M. whitei* and testosterone, however, did not show any significant changes when compared to the controls and testosterone-treated rats (Table 5).

4.2.2 EFFECT OF EXTRACT ON SOME REPRODUCTIVE STRUCTURES

4.2.2.1 Testes

Treatment of rats with *Mondia* root extract and testosterone was over three weeks. At the end of each week the weight of testes from sacrificed rats were measured. Changes in the weight of the testes weight that occurred during the first week of treatment among the untreated, testosterone-, *M. whitei*- and combined *M. whitei* and testosterone-treated albino rats were insignificant ($P>0.05$). Highly significant increases ($P< 0.001$) occurred

though within the third week among the combined *M. whitei* (2.05 mg/kg) and testosterone (1 mg/kg) treated rats when compared to the controls and testosterone-treated rats, whereas in the second week significant increases occurred in the combined *M. whitei* (4.11 mg/kg) and testosterone (1 mg/kg) treated rats when compared to testosterone treated and 2.05 mg/kg extract treated rats (Table 8).

Table 5 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on the average percentage increase in body weight over a period of 3 weeks. All values are mean ± SEM. ns P>0.05 not significant,* P<0.05, ** P<0.01, *** P<0.001 Significantly different from Control, @P<0.05, @@ P<0.01, Significantly different from testosterone-treated, ¨ P<0.01 Significantly different from 4.11 mg/kg *M. whitei*-treated group. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Comparisons Tests

Dose (mg/kg)	1st week Mean ± SEM	2nd week Mean ± SEM	3rd week Mean ± SEM
0 (Control)	7.83 ±1.37	19.55 ± 1.79	66.35 ± 4.83^{ns}
1 (Testosterone)	61.99 ± 5.97***	93.97 ± 8.66**	146.58 ± 21.80^{ns}
2.05 (*M. whitei*)	61.77 ± 8.05***	91.24 ± 10.38**	137.37 ± 18.07^{ns}
4.11 (*M. whitei*)	86.89 ± 6.88***	112.06 ± 14.41***	154.62 ± 77.87^{ns}
8.22 (*M. whitei*)	72.78 ± 8.56***	106.51 ± 13.05***	148.33 ± 19.22^{ns}
2.05(*M. whitei*)+1 (Testosterone)	33.88 ± 4.80^{ns}	45.91 ± 6.96¨	82.94 ± 22.73^{ns}
4.11(*M. whitei*)+1 (Testosterone)	30.61 ± 6.19@	62.42 ± 13.05¨	105.30 ± 17.65^{ns}
8.22(*M. whitei*)+1 (Testosterone)	25.69 ± 8.83@@	52.02 ± 13.25^{ns}	109.63 ± 44.69^{ns}

Table 6 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on the average weight of testes from 54 sacrificed rats over a period of 3 weeks. All values are mean ± SEM. ***Significantly different from Control P<0.001, @@ P<0.01, @@@ P<0.001 Significantly different from testosterone-treated, ¨ P<0.01 Significantly different from 2.05 mg/kg *M. whitei* group, ¨¨ P<0.001 Significantly different from 4.11 mg/kg *M. whitei*-treated group. All comparisons were done by one-way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Tests.

Dose (mg/kg)	1st week Mean ± SEM(g)	2nd week Mean ± SEM(g)	3rd week Mean ± SEM(g)
0 (Control)	0.67 ± 0.05	0.79 ± 0.03	0.57 ± 0.07
1 (Testosterone)	0.58 ± 0.06^{ns}	0.53 ± 0.04^{ns}	0.48 ± 0.002^{ns}
2.05 (*M. whitei*)	0.56 ± 0.05^{ns}	0.58 ± 0.01^{ns}	0.62 ± 0.02^{ns}
4.11 (*M. whitei*)	0.56 ± 0.05^{ns}	0.63 ± 0.02^{ns}	0.47 ± 0.05^{ns}
8.22 (*M. whitei*)	0.58 ± 0.05^{ns}	0.54 ± 0.05^{ns}	0.63 ± 0.01^{ns}
2.05(*M. whitei*)+1 (Testosterone)	0.66 ± 0.04^{ns}	0.72 ± 0.10^{ns}	0.89±0.66***.@@@.¨¨
4.11(*M. whitei*)+1 (Testosterone)	0.67 ± 0.04^{ns}	0.86 ± 0.10@@.¨	0.61 ± 0.33^{ns}
8.22(*M. whitei*)+1 (Testosterone)	0.67 ± 0.04^{ns}	0.56 ±0.03^{ns}	0.46 ± 0.02^{ns}

Over 3 weeks of treatments with testosterone (1 mg/kg) and that of the combined *M. whitei* (8.22 mg/kg) and testosterone-treated rats, microscopic slides and photomicrographs of the sections of the testes studied, showed some maturity of sperm cells. Normal spermatogenesis could be followed at the end of each week of treatment. There was a normal maturation of germ cells from the base of majority of the seminiferous tubules to the centre of their lumen. Numerous spermatogonia were seen adjoining the basement membrane, primary and secondary spermatocytes with spermatids and occasionally sperms (Plates 5D, 6D, 7D).

Compared to the 8.22 mg/kg *M. whitei*-treated rats (Plates 5C, 6C, 7C), histologically the testosterone (1 mg/kg) (Plates 5B, 6B, 7B) and combined treatments, photomicrographs showed more maturation (Plates 5D, 6D, 7D). But in the controls, in some of the seminiferous tubules, numerous spermatogonia and spermatocytes were seen, sometimes with a few spermatids indicating the initiation of spermatogenesis (Plates 5A, 6A, 7A). Occasionally some spermatids and sperm were observed in some of the seminiferous tubules of the 8.22 mg/kg *M. whitei*- treated rats (Plates 5C, 6C, 7C).

PLATE 5 SECTIONS OF TESTES OF RATS SACRIFICED AFTER ONE WEEK TREATMENT (X160)

Photomicrograph A is a cross section of the testes of a control rat stained with eosin showing seminiferous tubules (ST) containing healthy spermatogonia (SPG), both primary (PS) and secondary (SS) and a few spermatids (SPT) indicating the initiating of spermatogenesis in a few of the tubules.

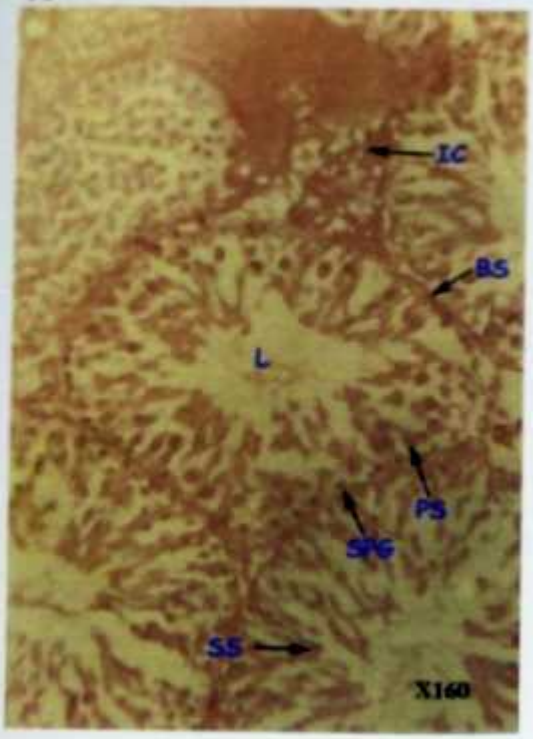
Photomicrograph B illustrates is a cross section of the testes of a testosterone-treated rat showing an orderly maturation of germ cells from the base membrane (BS) of the seminiferous tubules to the centre of the lumen (L). Some tubules showed spermatids (SPT) and mature sperm(S).

Photomicrograph C is a cross section of the testes of an 8.22 mg/kg (2x normal dose) *M. whitei*-treated rat. Spermatogenesis could be traced but fewer seminiferous tubules illustrated this. Spermatogonia (SPG), primary (PS) and secondary (SS) and occassional spermatids (SPT) and mature sperm (S) were observed.

Photomicrograph D shows a cross section of the testes of a combined treatment of 8.22 mg/kg (2x normal dose) *M. whitei* and 1 mg/kg testosterone-treated rat. Majority of seminiferous tubules showed maturity, illustrating healthy spermatogonia (SPG), primary (PS) and secondary (SS), spermatids (SPT) and mature sperm (S)

Plate 5 SECTIONS OF TESTES OF RATS SACRIFICED AFTER ONE WEEK TREATMENT (X160)

A



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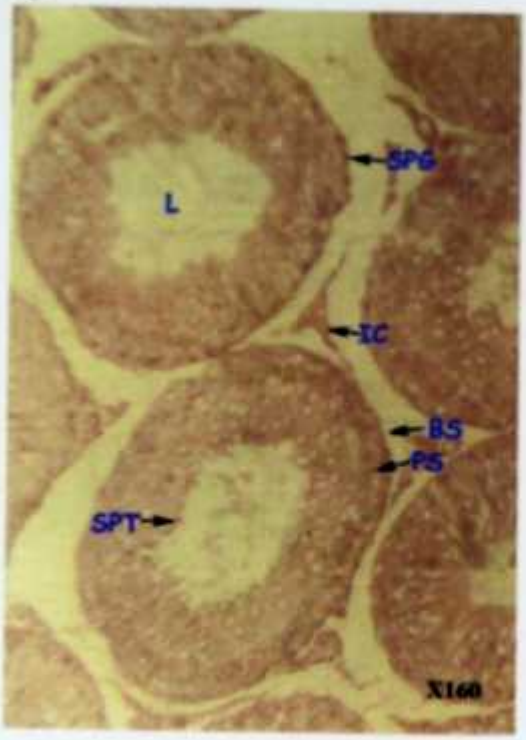

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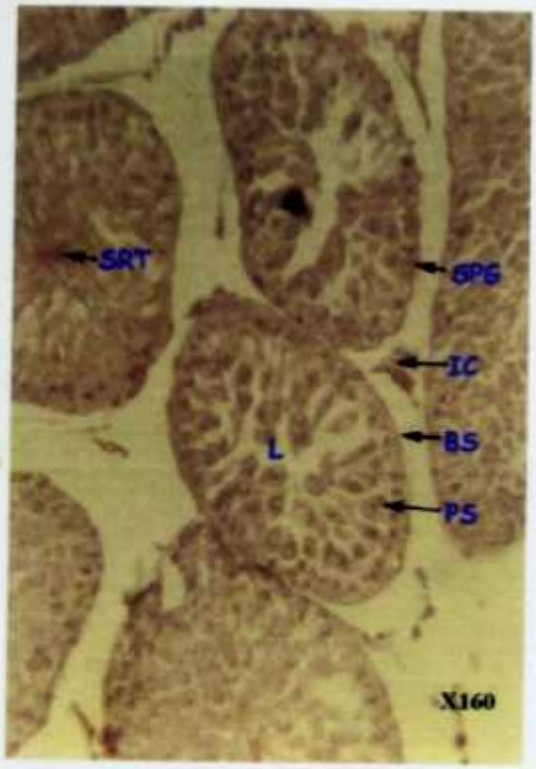

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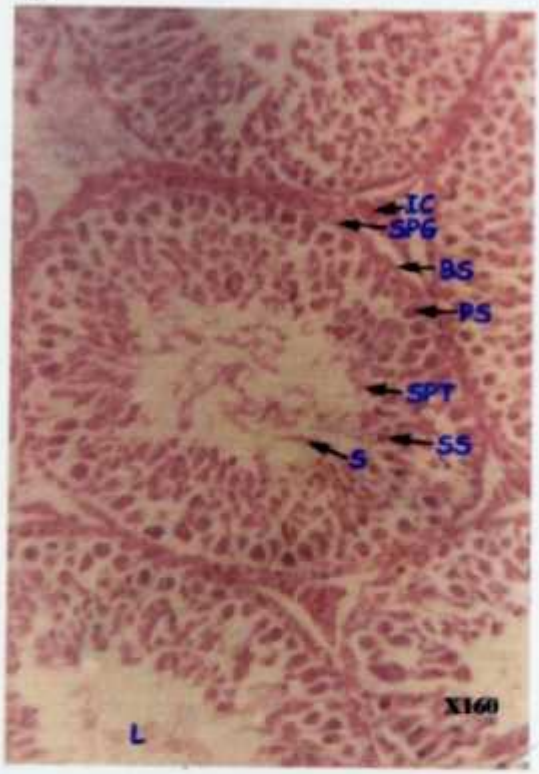


PLATE 6 SECTIONS OF TESTES OF RATS SACRIFICED AFTER TWO WEEKS TREATMENT (X160)

Photomicrograph A illustrates is a cross section of the testes of a control rat showing seminiferous tubules. Germ cells look healthy. Spermatogonia (SPG), primary (PS), secondary (SS) and spermatids (SPT) are seen from the base membrane (BS) to lumen (L). Mature sperm(S) are shown in some of the seminiferous tubules. Sections also show interstitial cells (IC).

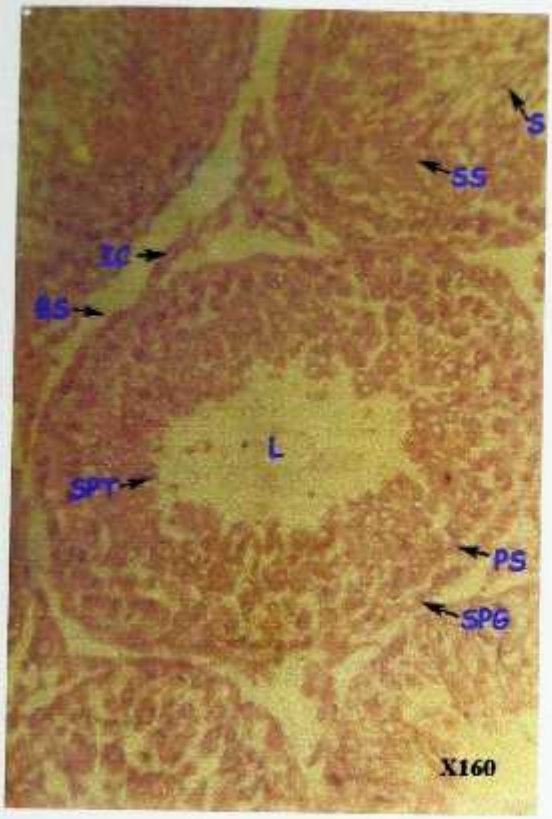
Photomicrograph B illustrates is a cross section of the testes of a testosterone-treated rat showing an orderly maturation of germ cells the base membrane (BS) of the seminiferous tubules to the centre of the lumen (L). Spermatids (SPT) and mature mature sperm (S) are seen in most of the seminiferous tubules.

Photomicrograph B illustrates maturation of germ cells, with healthy Spermatogonia (SPG), primary (PS), secondary (SS) and spermatids (SPT) are seen from the base membrane (BS) to lumen (L). This cross section of the testes of an 8.22 mg/kg (2x normal dose) *M. whitei*-treated rat shows more seminiferous tubules with spermatids (SPT) than sperm (S).

Photomicrograph D shows a cross section of the testes of a combined treatment of 8.22 mg/kg (2x normal dose) *M. whitei* and 1 mg/kg testosterone-treated rat. Seminiferous tubules show healthy spermatogonia (SPG), primary (PS) and secondary (SS), spermatids (SPT) and mature sperm (S) from the basement membrane (BS) to the lumen (L). All Photomicrographs show interstitial cells (IC).

Plate 6 SECTIONS OF TESTES OF RATS SACRIFICED AFTER TWO WEEKS TREATMENT (X160)

A



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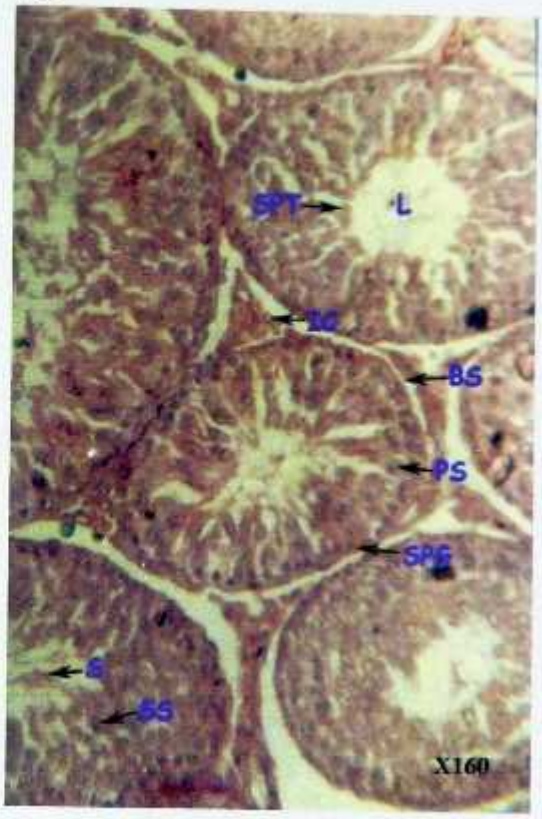

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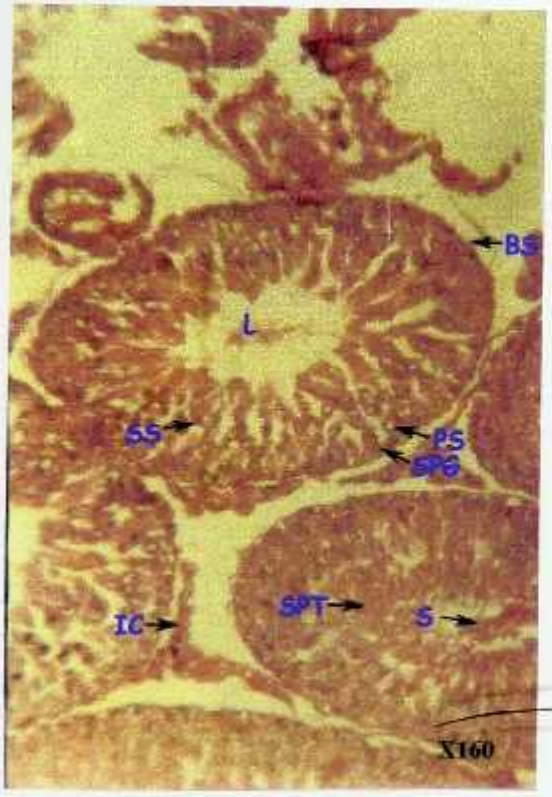

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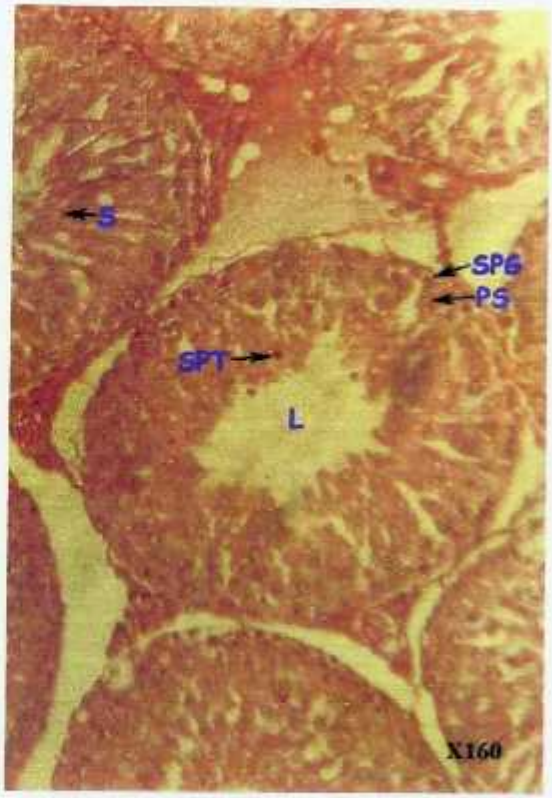


PLATE 7 SECTIONS OF TESTES OF RATS SACRIFICED AFTER THREE WEEKS TREATMENT (X160)

Photomicrograph A is a cross section of the testes of a control rat stained with eosin showing seminiferous tubules (ST) with fewer spermatogenic elements. Healthy spermatogonia (SPG) are seen along the basement membrane. Some tubules contain primary (PS) and secondary (SS) and a few spermatids (SPT) indicating the initiating of spermatogenesis in a few of the tubules.

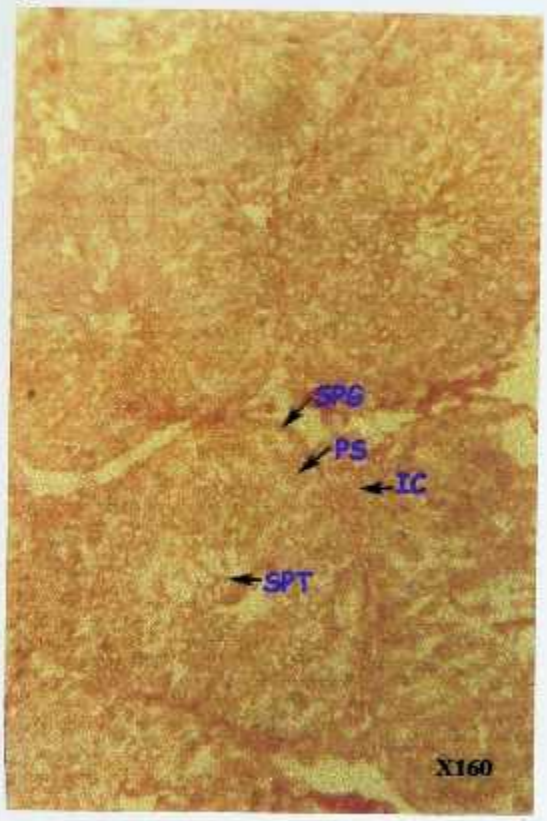
Photomicrograph B illustrates is a cross section of the testes of a testosterone-treated rat showing an orderly maturation of germ cells from the base membrane (BS) of the seminiferous tubules to the centre of the lumen (L). Spermatogenesis could be well traced but showing healthy spermatogonia (SPG), primary (PS) and secondary (SS), spermatids (SPT) and mature sperm (S).

Photomicrograph C is a cross section of the testes of a 8.22 mg/kg (2x normal dose) *M .whitei*-treated rat. Spermatogenesis could be traced but fewer seminiferous tubules illustrated this. Spermatogonia (SPG) and primary (PS) are very prominent. Occasional secondary (SS) and spermatids (SPT) and were observed.

Photomicrograph D shows a cross section of the testes of a co-joint treatment of 8.22 mg/kg (2x normal dose) *M. whitei* and 1 mg/kg testosterone treated rat. Majority of seminiferous tubules showed maturity, illustrating healthy spermatogonia (SPG), primary (PS) and secondary (SS), spermatids (SPT) and mature sperm (S).

Plate 7 SECTIONS OF TESTES OF RATS SACRIFICED AFTER THREE WEEKS TREATMENT (X160)

A



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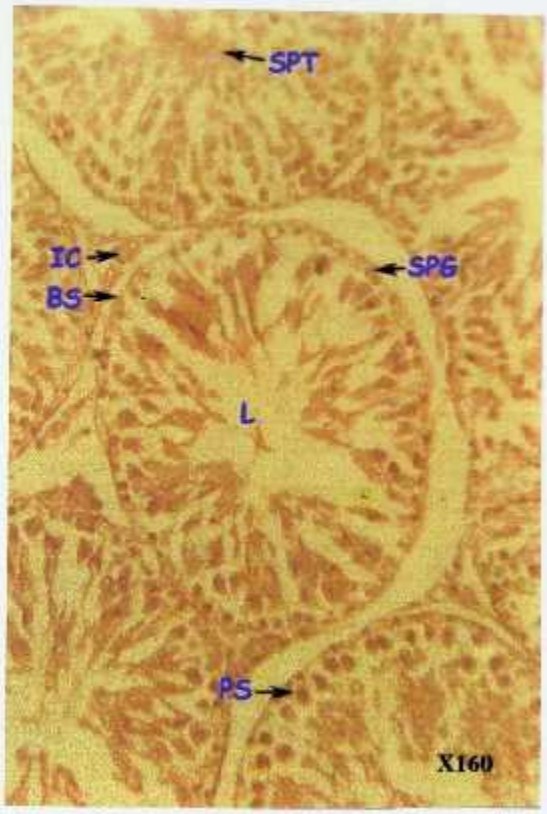

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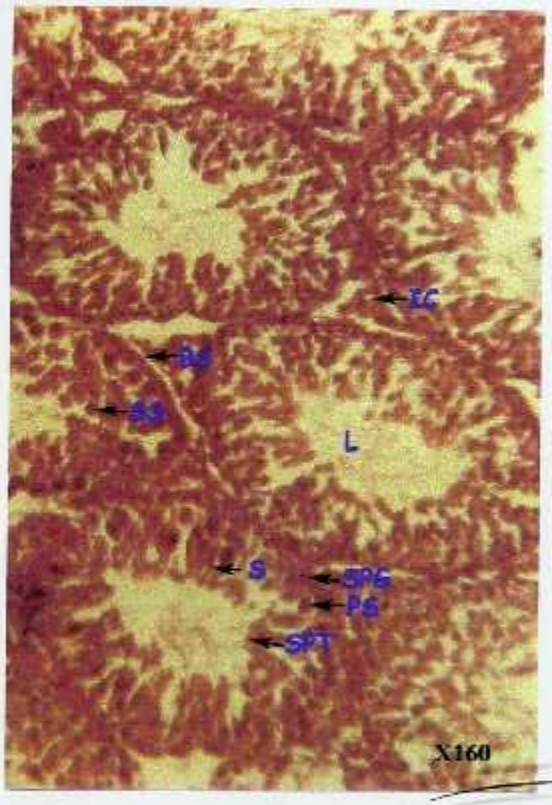

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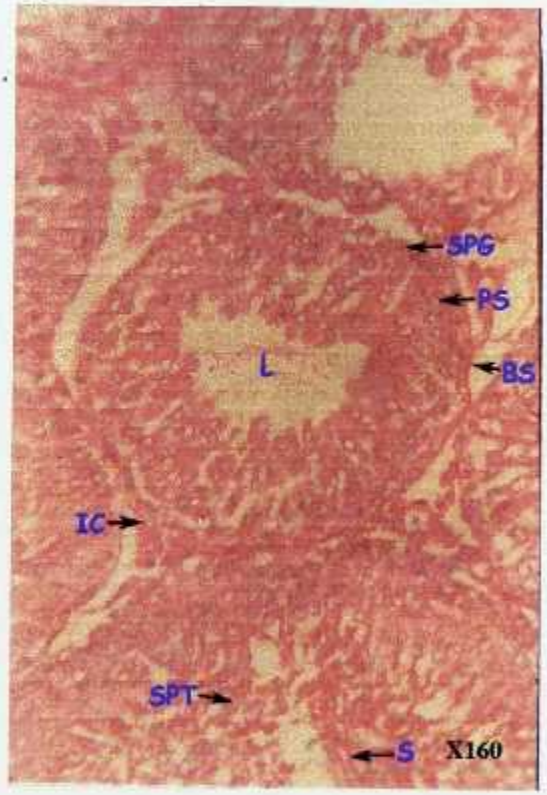


4.2.2.2 Epididymis

Epididymis from each animal (rat) that was treated with either testosterone (1 mg/kg) or with the *Mondia* extract alone at 2.05 mg/kg, 4.11 mg/kg or 8.22 mg/kg dose levels were weighed and any change in weight observed. Rats with combined treatments were also sacrificed each week had their epididymis also weighed. Throughout the three weeks of observation of epididymal weights, *Mondia* extract dose level (2.05 mg/kg) + testosterone (1 mg/kg) recorded the largest significant increase in weight ($P<0.001$). After the first and third weeks of oral dosing 2.05 mg/kg *Mondia* combined with 1 mg/kg testosterone compared to the controls recorded a very significant increase, $P<0.001$. No significant changes occurred with the 2.05 mg/kg, 4.11 mg/kg and 8.22 mg/kg doses of *M. whitei* compared to the controls and the testosterone-treated rats (Table 7).

4.2.2.3 Seminal Vesicles

Each organ (seminal vesicles) that was taken from sacrificed rats at the end of each one of the 3 weeks of treatment, was weighed before fixing in Bouins fluid. Percentage organ weights of controls, 2.05 mg/kg, 4.11 mg/kg and 8.22 mg/kg *M. whitei*-treated rats as well as 2.05 mg/kg *M. whitei* + 1 mg/kg testosterone, 4.11 mg/kg *M. whitei* + 1 mg/kg testosterone and 8.22 mg/kg *M. whitei* + 1 mg/kg testosterone treated rats were compared. Most weight changes occurred during the 1st week of treatment. *M. whitei* -treated (*M. whitei)* rats at all the dose levels showed highly significant decreases at ($P\leq0.001$) in seminal vesicles weights when compared to the testosterone-treated ones, but showed no changes when compared to the untreated. This could be indicative of the fact that *M. whitei* did not froduce any change. Comparisons between dose levels of *M. whitei* (2.05

mg/kg, 4.11 mg/kg and 8.22 mg/kg), controls and testosterone-treated rats showed no significant changes ($P>0.05$) within the 2nd week of treatment. However a highly significant increase that occurred in the third week of administration were those that occurred with the simultaneous 2.05 mg/kg *M. whitei* and 1 mg/kg of testosterone-treated organ weights ($P<0.001$). The combined treatment of 8.22 mg/kg *M. whitei* and 1 mg/kg testosterone also caused quite a significant increase, ($P<0.001$) when compared to the controls (Table 8).

Statistical evidence (Table 9) indicate that no significant changes also occurred with the relative epithelial lining widths of the seminal vesicles in these rats within the first 2 weeks. A significant increase only occurred after 3 weeks with glandular epithelial lining of the 8.22 mg/kg *M. whitei*-treated when compared to the controls. A complex papillary folds and irregular branching lumina of seminal vesicular mucosa is evident. Cells of the glandular epithelia had prominent nucleoli embedded in large nuclei. Histological evidence can be seen in Plates 9E-9F.

Table 7 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on average weight of epididymis from 54 sacrificed rats over a period of 3 weeks. All values are mean ± SEM. * P<0.05, *** P<0.001 Significantly different from Control, @ P<0.05, @@@ P<0.001 Significantly different from testosterone-treated, ''' P<0.001 Significantly different from 3 doses of *M. whitei*-treated groups. All comparisons were done by one-way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Tests.

Dose (mg/kg)	1st week Mean ± SEM(g)	2nd week Mean ± SEM(g)	3rd week Mean ± SEM(g)
0 (Control)	0.09 ± 0.01	0.16 ± 0.02	0.09 ± 0.02
1 (Testosterone)	0.15 ± 0.02*	0.12 ± 0.01^{ns}	0.11 ± 0.01^{ns}
2.05 (*M. whitei*)	0.04 ± 0.01^{ns}	0.13 ± 0.02^{ns}	0.12 ± 0.01^{ns}
4.11 (*M. whitei*)	0.10 ± 0.02^{ns}	0.14 ± 0.01^{ns}	0.09 ± 0.01^{ns}
8.22 (*M. whitei*)	0.10 ± 0.01^{ns}	0.10 ± 0.01^{ns}	0.12 ± 0.01^{ns}
2.05(*M. whitei*)+1(Testosterone)	0.21 ± 0.01***.@	0.17 ± 0.03^{ns}	0.21±0.01***.@@@,'''
4.11(*M. whitei*)+1(Testosterone)	0.10 ± 0.01@	0.20 ± 0.01^{ns}	0.12 ± 0.01^{ns}
8.22(*M. whitei*)+1(Testosterone)	0.13 ± 0.01^{ns}	0.13 ± 0.01^{ns}	0.14 ± 0.01^{ns}

Table 8 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on average weight of seminal vesicles from 54 sacrificed rats over a period of 3 weeks. All values are mean ± SEM. ns P>0.05 not significant, ** P<0.01, *** P<0.001 Significantly different from Control, @@@ P<0.001 Significantly different from testosterone. All comparisons were done by one-way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Tests

Dose (mg/kg)	1st week Mean ± SEM(g)	2nd week Mean ± SEM(g)	3rd week Mean ± SEM(g)
0 (Control)	0.043 ± 0.004	0.148 ± 0.029	0.102 ± 0.054
1 (Testosterone)	0.396 ± 0.199***	0.369± 0.087^{ns}	0.137 ± 0.020^{ns}
2.05 (*M. whitei*)	0.099 ± 0.04@@@	0.155± 0.066^{ns}	0.155 ± 0.063^{ns}
4.11 (*M. whitei*)	0.151 ± 0.045@@@	0.165± 0.050^{ns}	0.079 ± 0.030^{ns}
8.22 (*M. whitei*)	0.074 ± 0.009@@@	0.101±0.030^{ns}	0.177 ± 0.051^{ns}
2.05(*M. whitei*)+1 (Testosterone)	0.408 ± 0.019***	0.431± 0.171^{ns}	0.719±0.004***.@@@
4.11(*M. whitei*)+1 (Testosterone)	0.073 ± 0.008@@@	0.526± 0.076^{ns}	0.316 ± 0.016^{ns}
8.22(*M. whitei*)+1 Testosterone)	0.183±0.018**.@@@	0.245± 0.086^{ns}	0.419 ± 0.109**

Table 9 The effect of *M. whitei* and testosterone on the average widths of glandular epithelial lining of Seminal vesicles from 54 rats sacrificed over a period of 3 weeks. All values are mean ± SEM. ns P>0.01 not significant, * P<0.05 Significantly different from control. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Comparisons Tests

Dose (mg/kg)	1st week Mean ± SEM	2nd week Mean ± SEM	3rd week Mean ± SEM
0 (Control)	0.020± 0.001	0.020 ± 0.001	0.019 ± 0.001
1 (Testosterone)	0.021± 0.002^{ns}	0.022 ± 0.001^{ns}	0.020 ± 0.001^{ns}
2.05 (Extract)	0.023 ± 0.001^{ns}	0.019 ± 0.001^{ns}	0.022 ± 0.002^{ns}
4.11 (Extract)	0.025 ± 0.001^{ns}	0.026 ± 0.002^{ns}	0.021 ± 0.001^{ns}
8.22 (Extract)	0.023 ± 0.001^{ns}	0.023 ± 0.001^{ns}	0.026 ± 0.002*

After studying the microscopic slides, the photomicrographs taken of testosterone-treated rats throughout the 3 weeks of treatment showed very fluid filled lumina of the seminal vesicles. After 1 week of treatment, compared to the controls (Plate 8A), rats treated with 2.05 mg/kg (Plate 8C), 4.11 mg/kg (Plate 8D), and 8.22 mg/kg (Plate 8E) of the *M. whitei* extract, showed more fluid filled lumina. Histological sections looked normal, with the complex papillary folds and irregular branching lumina of seminal vesicular mucosa evident. Cells of the glandular epithelia had prominent nucleoli embedded in large nuclei. Muscular layers were observed in certain parts in majority of the photomicrographs taken. Seminal vesicles of rats with combined treatments had more fluid filled lumina than that of the controls. Also 8.22 mg/kg *M. whitei* and 1 mg/kg of testosterone treated rats (Plate 8F) looked normal with papillary folds and irregular branching lumina as controls but were more fluid filled in the lumina spaces than the controls.

Histological sections of the seminal vesicles of rats treated with *M. whitei* (Plate 9C) and testosterone (Plate 9B) showed lumina that were fluid filled when compared to the controls (Plate 9A). The sections seminal vesiclesof rats treated with 2.05 mg/kg *M. whitei* and 1 mg/kg of testosterone (Plate 9D) looked normal with the complex papillary folds and irregular branching lumina of seminal vesicular mucosa evident and were more fluid filled in the lumina spaces than the controls.

PLATE 8 SEMINAL VESICLES OF RATS SACRIFICED AFTER ONE WEEK OF TREATMENT (X100)

Photomicrograph A illustrates a cross section of seminal vesicles of a control rat showing the folding of its mucosa and lumen (L). The epithelial layer (EP) has columnar cells with prominent centrally located nucleus (N). Muscular layer (M) can also be seen.

Photomicrograph B illustrates a cross section of seminal vesicles of a testosterone-treated rat. At a low magnification, the complex papillary folds and irregular branching of the lumena of seminal vesicular mucosa are evident. The lumen (L) is very fluid filled with seminal fluid.

Photomicrograph C, at a low magnification, illustrates a cross section of seminal vesicles of a 2.05 mg/kg *M. whitei*-treated rat. The epithelial layer (EP) has columnar cells with prominent centrally located nucleus (N). Muscular layer (M) can also be seen. Lumen (L) is fluid filled.

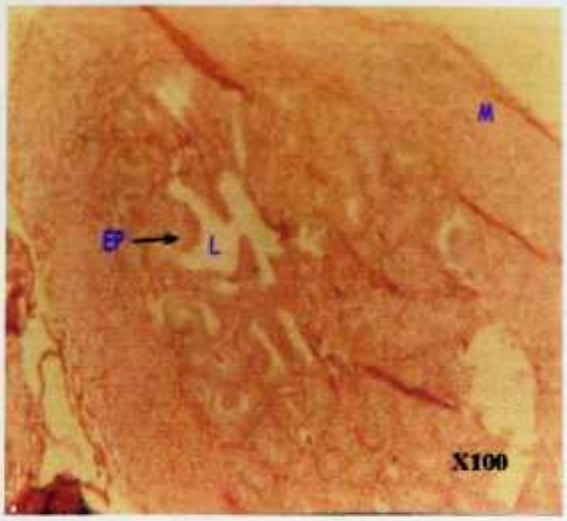
Photomicrograph D illustrates a cross section of seminal vesicles of a 4.11 mg/kg *M. whitei*-treated rat. At a low magnification, the complex papillary folds and irregular branching of the lumena of seminal vesicular mucosa are evident. The lumen (L) is very fluid filled with seminal fluid.

Photomicrograph E shows a cross section of seminal vesicles of an 8.22 mg/kg *M. whitei*-treated rat. With very fluid filled lumen (L), the complex papillary folds and irregular branching of the lumena of seminal vesicular mucosa are also evident.

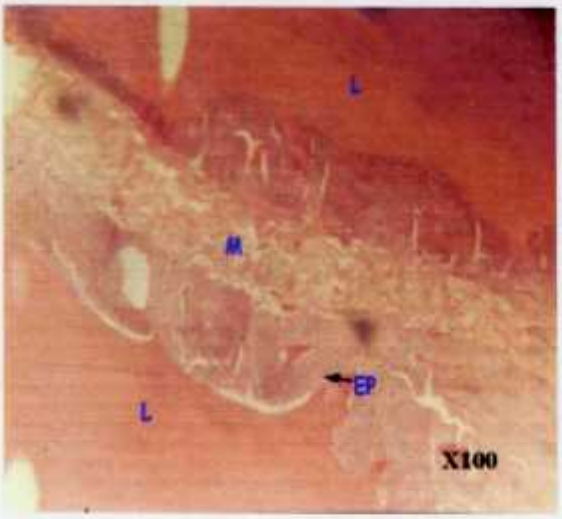
Photomicrograph F is a cross section of seminal vesicles at a lower magnification of a combined treatment of a dose of 8.22 mg/kg *M. whitei* and 1 mg/kg testosterone-treated rat. The epithelial layer (EP) has columnar cells with prominent centrally located nucleus (N). Muscular layer (M) can also be seen. Lumen (L) is fluid filled.

Plate 8 SEMINAL VESICLES OF RATS SACRIFICED AFTER ONE WEEK OF TREATMENT(X100)

A



D



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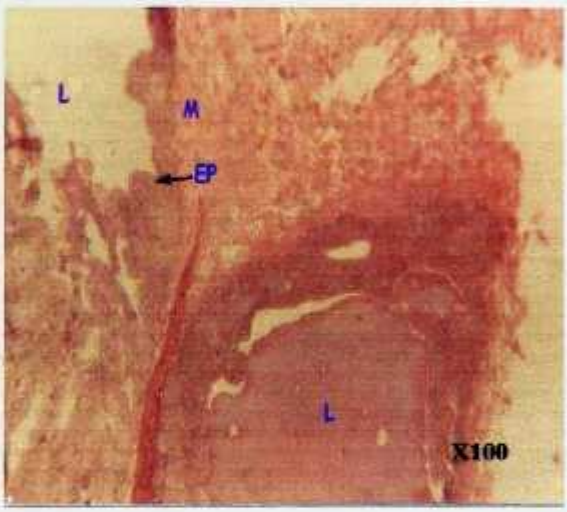

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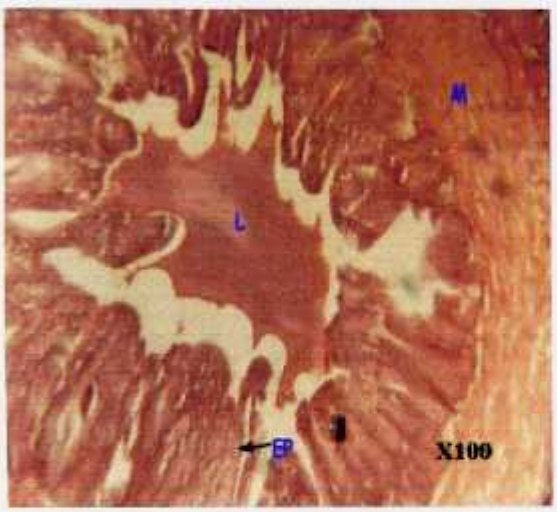
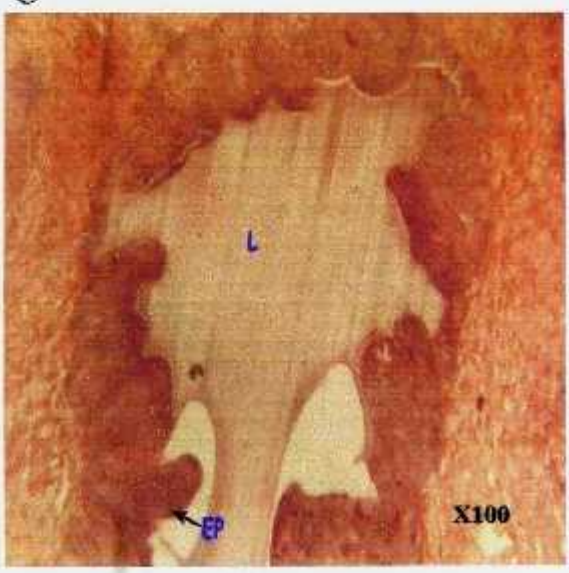


C



F

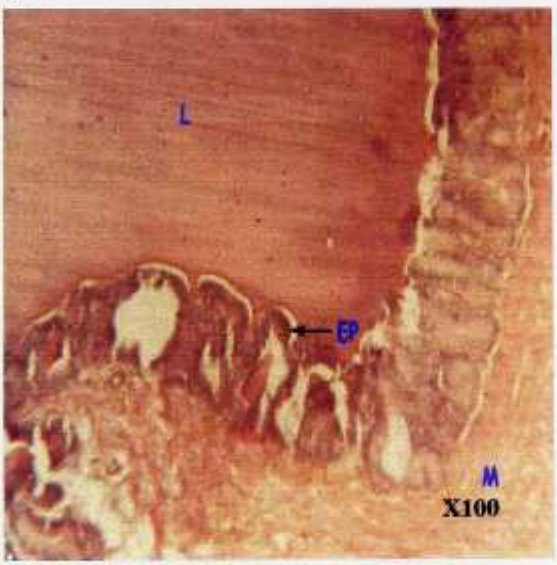


PLATE 9 SEMINAL VESICLES OF RATS SACRIFICED AFTER THREE WEEKS OF TREATMENT (X100)

Photomicrograph A illustrates a cross section of seminal vesicles of a control rat showing the folding of its mucosa and lumen (L). The epithelial layer (EP) has columnar cells with prominent centrally located nucleus (N). Muscular layer (M) can also be seen.

Photomicrograph B illustrates a cross section of seminal vesicles of a testosterone-treated rat. At a low magnification, the complex papillary folds and irregular branching of the lumena of seminal vesicular mucosa are evident. The lumen (L) is quite fluid filled with seminal fluid.

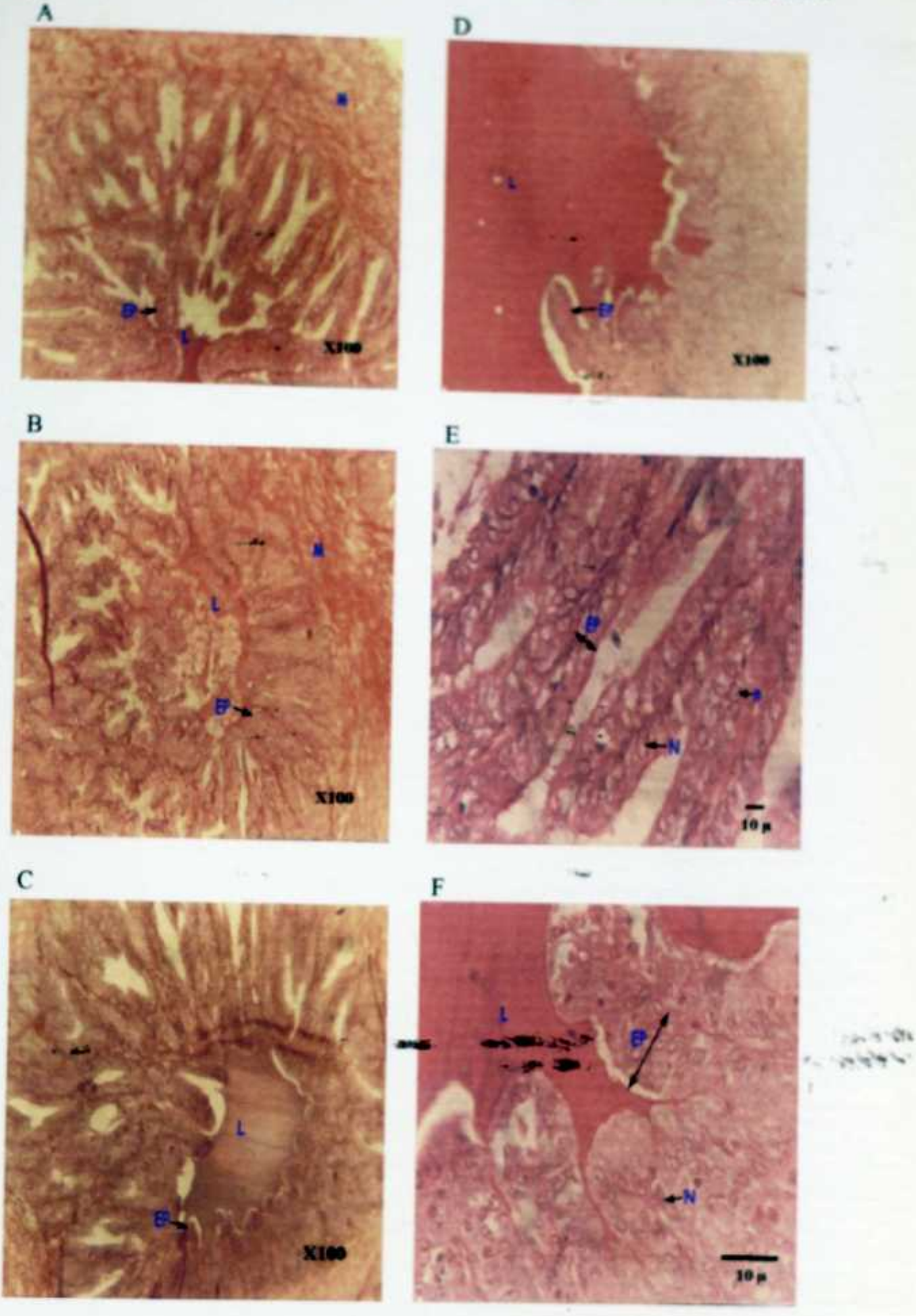
Photomicrograph C illustrates a cross section of seminal vesicles of an 8.22 mg/kg *M.whitei*-treated rat. At a low magnification, the complex papillary folds and irregular branching of the lumena of seminal vesicular mucosa are evident. The lumen (L) is very fluid filled with seminal fluid.

Photomicrograph D is a cross section of seminal vesicles at a lower magnification of a combined treatment of a dose of 2.05 mg/kg *M. whitei* and 1 mg/kg testosterone-treated rat. The epithelial layer (EP) has columnar cells with prominent centrally located nucleus (N). Muscular layer (M) can also be seen. Lumen (L) is fluid filled.

Photomicrograph E at a higher magnification shows a cross section of seminal vesicles of a control rat, showing a less thicker width of epithelial lining (EP). Secretory epithelial cells look normal with large prominent nucleus (N) and nucleolus (n) in some.

Photomicrograph F illustrates a cross section of seminal vesicles of an 8.22 mg/kg *M. whitei*-treated rat. At a high magnification, lumen (L) is fluid filled with tall columnar secretory epithelial cells that have clear nuclei and nucleolus. The epithelial layer (EP) shows a larger width.

Plate 9 SEMINAL VESICLES OF RATS SACRIFICED AFTER THREE WEEKS OF TREATMENT (X100)



4.2.2.4 Ventral Prostate

At the end of every week of the experimental period, ventral prostate organs which were removed from the control rats, testosterone-treated rats, 2.05 mg/kg, 4.11 mg/kg and 8.22 mg/kg *M. whitei*-treated rats as well as 2.05 mg/kg *M. whitei* + 1 mg/kg testosterone, 4.11 mg/kg *M. whitei* + 1 mg/kg testosterone and 8.22 mg/kg *M. whitei* + 1 mg/kg testosterone-treated rats were all weighed. Their weights were calculated with respect to the body weights of rats from which they were removed.

During the first week of administration (Table 10), the testosterone treated prostate compared to the controls, showed a significant increase, $P<0.05$ in organ weight as well as a very significant increase in relative epithelial lining widths throughout the 3 week period. Dose levels of 4.11 mg/kg and 8.22 mg/kg of *M. whitei* caused significant organ weight increase at $P<0.01$ and $P<0.05$ respectively compared to the controls after the 1st week of oral dosing. Dose 8.22 mg/kg *M. whitei* also resulting in a highly significant epithelial width ($P<0.001$). Histological evidence of increase in epithelial width of lining of ventral prostate of the 8.22 mg/kg dose level of *M. whitei* compared to the controls can be seen in Plates 10F and 10E respectively. Even though a dose level of 2.05 mg/kg *M. whitei* did not cause any significant ($P>0.05$) changes in organ weight, there was a very significant decrease in the epithelial width when compared to testosterone-treated, $P<0.001$, and an increase from the controls, $P<0.001$ (Table 11). Histologically, Plate 10G illustrates this highly significant decrease in epithelial width of the 2.05 mg/kg *M. whitei*-treated ventral prostate organs when compared to the testosterone-treated (Plate 10F). The change observed between the epithelial glandular linings of the 2.05 mg/kg *M.*

whitei-treated rats compared to the controls can be seen in Plate 10E. Histological evidence can be seen in Plates 10F (testosterone-treated), 10G (2.05 mg/kg *M. whitei*-treated), 10H (8.22 mg/kg *M. whitei*-treated) and Plate 10E (controls). After the 3rd week, all combined treatments showed significant increases in organ weight and there was no net change in ventral prostate weight with the *M. whitei* treatments (Table 10).

Table 10 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on average weight of Ventral Prostate from 54 sacrificed rats over a period of 3 weeks. All values are mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001 Significantly different from Control, @@@ P<0.001 Significantly different from testosterone-treated groups. All comparisons were done by one-way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Tests

Dose (mg/kg)	1st week Mean ± SEM(g)	2nd week Mean ± SEM(g)	3rd week Mean ± SEM(g)
0 (Control)	0.023 ± 0.006	0.051 ± 0.004	0.057 ± 0.027
1 (Testosterone)	0.127 ± 0.030*	0.113 ± 0.019^{ns}	0.027 ± 0.003^{ns}
2.05 (*M. whitei*)	0.064 ± 0.026^{ns}	0.060 ± 0.011^{ns}	0.055 ± 0.021^{ns}
4.11 (*M. whitei*)	0.144 ± 0.021**	0.071 ± 0.021^{ns}	0.041 ± 0.005^{ns}
8.22 (*M. whitei*)	0.124 ± 0.023*	0.080± 0.040^{ns}	0.043 ± 0.007^{ns}
2.05(*M. whitei*)+1 (Testosterone)	0.137 ± 0.002**	0.124 ± 0.051^{ns}	0.175± 0.023**.@@@
4.11(*M. whitei*)+1 (Testosterone)	0.043 ± 0.008^{ns}	0.112 ± 0.019^{ns}	0.316 0.016***.@@@
8.22(*M. whitei*)+1 (Testosterone)	0.058 ± 0.007^{ns}	0.087 ± 0.030^{ns}	0.145 ± 0.008*.@@@

Table 11 The effect of *M. whitei* and testosterone on the average widths of glandular epithelial lining of Ventral Prostate of 54 rats sacrificed over a period of 3 weeks. All values are mean ± SEM. ** P<0.01, *** P<0.001 Significantly different from control, @P<0.05, @@@ P<0.001 Significantly different from testosterone-treated groups. All comparisons were done by one-way analysis of variance (ANOVA), followed by Bonferroni Multiple Comparisons Tests.

Dose (mg/kg)	1st week Mean ± SEM	2nd week Mean ± SEM	3rd week Mean ± SEM
0 (Control)	0.009 ± 0.002	0.013 ± 0.002	0.013± 0.001
1 (Testosterone.)	0.019 ± 0.0001***	0.020± 0.001**	0.022 ± 0.002***
2.05 (*M. whitei*)	0.010 +0.001***,@@@	0.015= 0.001ns	0.015 ± 0.002 @
4.11 (*M. whitei*)	0.020 ± 0.001ns	0.016± 0.001ns	0.016 ± 0.001ns
8.22 (*M. whitei*)	0.022 ± 0.002***	0.015 ± 0.001ns	0.018 ± 0.001ns

A careful study of the microscopic slides and the photomicrographs of Ventral Prostate showed that there were sections with well defined pseudostratified columnar cells with normal stained nuclei and well formed septae for some of the controls as well as testosterone and drug treated rats. Organ slides of testosterone, *M. whitei* and combined treatments presented more fluid filled lobules compared to the controls. After 1 week of drug administration, *M. whitei* at dose levels of 4.11 mg/kg (Plate 10C) and 8.22 mg/kg(Plate 10D) and 1 mg/kg testosterone(Plate 10B), histological sections of the ventral prostate of these treated rats showed a lot of fluid filled lobules when compared to the controls (Plate 10A). For the last 2 weeks of drug administration even though no changes in organ weights were reported, it was observed histologically in Plate 11C, that the testosterone-treated rats showed majority of the lobules in the ventral prostate as fluid filled when compared to the controls (Plate 11A). Treatment with a dose level of 8.22

mg/kg *M. whitei* also showed some fluid filled cavities (Plate 11D). One of the rats histologically illustrated a quite undeveloped ventral prostate, with a lot of muscular layer seen and some deposits that could be calcified deposits (Plate 11B). Highly significant changes in ventral prostate weights were observed for the combined treatments after 3 weeks of treatment, so histologically sections of these organs were studied. Histological evidence showed that all the combined treatments (Plates 11F-H) illustrated well developed pseudostratified columnar cells and well formed septae. Lobules of these treatments showed fluid filled cavities when compared to the controls (Plate 11E).

PLATE 10 CROSS SECTIONS OF VENTRAL PROSTATE GLANDS OF RATS SACRIFICED AFTER ONE WEEK OF TREATMENT SHOWING LUMINA (L) ENCLOSED IN SEPTA (S).

Photomicrographs A and B illustrate the prostatic glandular tissue of a control rat and a 1mg/kg testosterone-treated rat respectively. (X100)

Photomicrographs C and D illustrate the prostatic glandular tissue of a typical *M. whitei* extract-treated rat at a dose of 4.11 mg/kg (normal dose) and 8.22mg/kg (twice the normal dose) respectively. (X100)

Photomicrographs E and F illustrate the prostatic glandular tissue of a control rat and a 1 mg/kg testosterone-treated rat respectively at higher magnifications (x400).

Photomicrographs G and H illustrate the prostatic glandular tissue of a typical *M. whitei* extract-treated rat at a dose of 2.05 mg/kg (normal dose) and 8.22 mg/kg (twice the normal dose) respectively (x400).

Complete septae are seen in all histological sections with large irregular lumina (L) that are more fluid filled for the testosterone and *M. whitei*-treated tissues. Widths of the glandular columnar epithelial lining (EP) for the treated rats (testosterone and *M. whitei*) are wider. Nuclei are large and basally located with prominent nucleoli and a wider glandular epithelial width compared to the controls.

Plate 10 CROSS SECTIONS OF VENTRAL PROSTATE GLANDS OF RATS SACRIFICED AFTER ONE WEEK OF TREATMENT SHOWING LUMINA (L) ENCLOSED IN SEPTA (S).

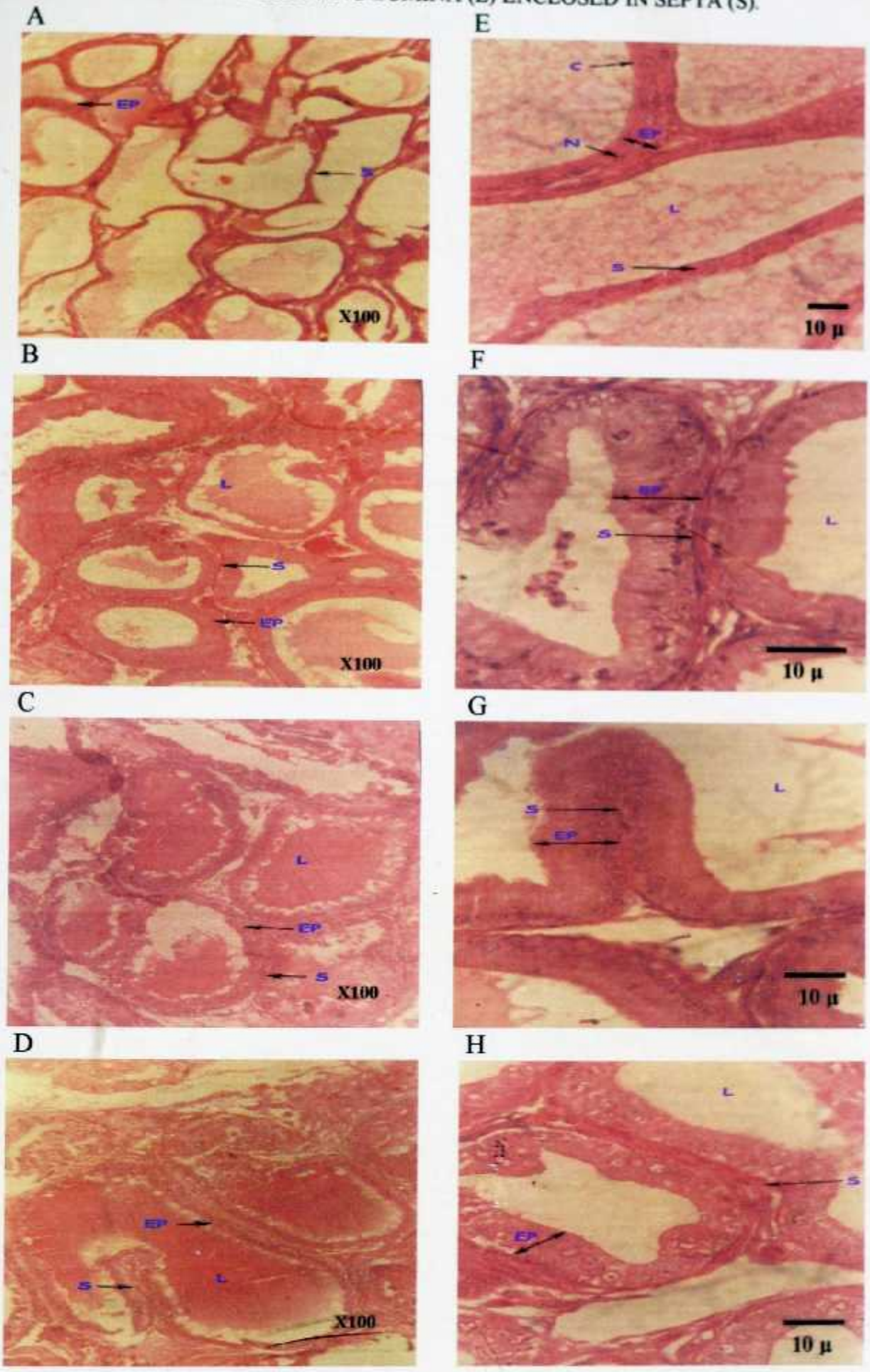


PLATE 11 CROSS SECTIONS OF VENTRAL PROSTATE GLANDS OF RATS SACRIFICED AFTER TREATMENT SHOWING LUMINA (L) ENCLOSED IN SEPTA (S). (X100)

Photomicrographs A and B illustrate the prostatic glandular tissue of control rats after two weeks of observation.

Photomicrographs C and D illustrate the prostatic glandular tissue of a typical testosterone-treated rat and a *M. whitei* extract-treated rat at 8.22 mg/kg (twice the normal dose) respectively. Treatment was for two weeks.

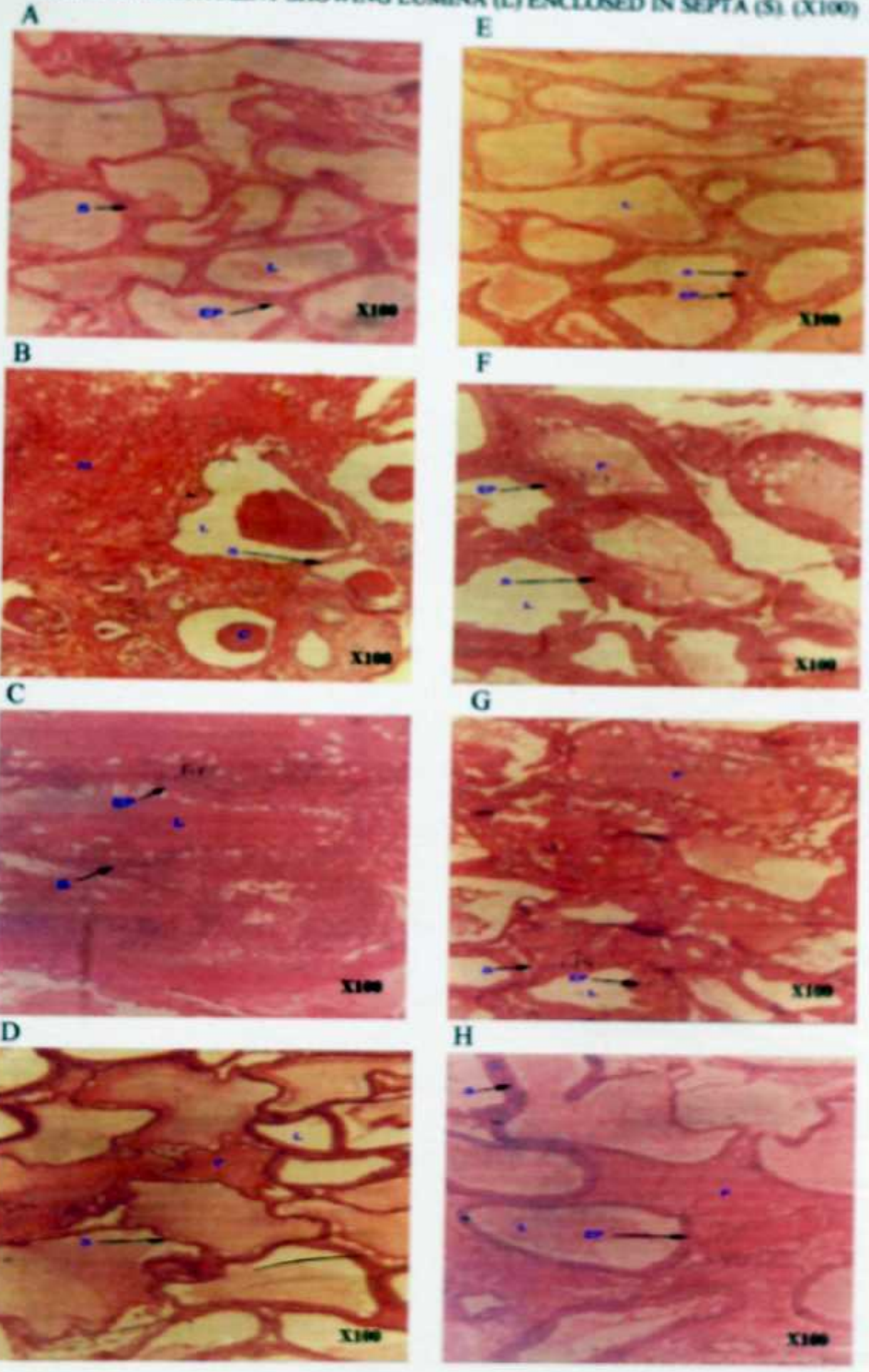
Complete septae (S) are shown in A, with B not as well developed with distinguish septae as seen in A. B shows a lot of muscular fibres (M) running in various directions. Lumina (L) contain lamella bodies called prostatic concretions (C). Epithelium (EP) is flattened to cuboidal type. C and D illustrate fluid filled lumina and thicker epithelial lining for C.

Photomicrographs E and F illustrate the prostatic glandular tissue of a control rat and a combination of 2.05 mg/kg *M. whitei* (normal dose) and 1 mg/kg testosterone-treated rat respectively. Treatment was for two weeks.

Photomicrographs G and H illustrate the prostatic glandular tissue of a combination of 4.11 mg/kg *M. whitei* (normal dose) and 1 mg/kg testosterone-treated rat and a combination of 8.22 mg/kg *M. whitei* (normal dose) and 1 mg/kg testosterone-treated rat respectively. Treatment was for two weeks.

Lumina (L) of combined treatments (F,G,H) are fluid filled with majority of portions of the sections showing wider glandular epithelial lining widths (EP), which are made of columnar cells. Lunmia of all sectons are irregular.

Plate 11 CROSS SECTIONS OF VENTRAL PROSTATE GLANDS OF RATS SACRIFICED AFTER 2 WEEKS OF TREATMENT SHOWING LUMINA (L) ENCLOSED IN SEPTA (S). (X100)



4.2.2.5 Vas Deferens

After each week of treatments, rats in each group of the different treatments were sacrificed, vas deferens freed from all fat deposits and weighed. Comparing organ weights, a highly significant increase in vas deferens weight of the testosterone-treated rat occurred by the end 3 weeks of dosing ($P<0.05$). The only significant change in the 1st week that was observed with respect to different dose levels of *M. whitei* that were used was a decrease when compared to the testosterone-treated, and no change comparing to controls (Table 14).

Within the subsequent weeks of treatment no significant changes were recorded when the 2.05 mg/kg, 4.11 mg/kg and 8.22 mg/kg dose levels of *M. whitei* was compared to the controls. When compared to the testosterone-treated rats, the vas deferens weights showed significant decreases in the three dose levels of *M. whitei* treated rats, within each week of observation. During the 2nd week a significant decrease ($P\leq0.05$) in organ weight was observed for dose levels of 2.05 mg/kg and 4.11 mg/kg *M. whitei*, whiles at a dose level of 8.22 mg/kg the decrease was quite significant ($P\leq0.01$) as compared to the testosterone-treated rats. By the end of the 3rd week only a dose level of 4.11 mg/kg *M. whitei* showed a decrease in vas deferens weight when compared to the testosterone-treated ($P\leq0.01$). Two weeks of oral extract dosing caused significant decreases in weight of vas deferens compared to testosterone-treated rats.

Combined treatments also showed significantly higher weights when compared to the controls and the dose levels of *M. whitei* treatments. Compared to the untreated,

combined treatments showed increases in the third week (Table 14). 2.05 mg/kg *M. whitei* + 1 mg/kg testosterone and 8.22 mg/kg *M. whitei* + 1 mg/kg testosterone dose levels showed highly significant increases in organ weight in the 1st and 3rd weeks of treatments when compared to the controls (P≤0.001). An oral dosing of 4.11 mg/kg *M. whitei* + 1 mg/kg testosterone showed significant increases in organ weight both by the end of the 1st and 3rd weeks also (P≤0.05) (Table 14)

Table 12 The effect of *M. whitei*, testosterone, *M. whitei* + testosterone on the average weight of Vas Deferens from 54 sacrificed rats over a period of 3 weeks. All values are mean ± SEM. * P<0.05, *** P<0.001 Significantly different from Control, @ P<0.05, @@ P<0.01, @@@ P<0.001 Significantly different from testosterone-treated groups. All comparisons were done by one-way analysis of variance (ANOVA), followed by Tukey-Kramer Multiple Comparisons Tests.

Dose (mg/kg)	1st week Mean ± SEM(g)	2nd week Mean ± SEM(g)	3rd week Mean ± SEM(g)
0 (Control)	0.026 ± 0.001	0.041 ± 0.005	0.028 ±0.004
1 (Testosterone)	0.042 ± 0.003***	0.050 ± 0.002^{ns}	0.039 ± 0.003^{ns}
2.05 (*M. whitei*)	0.028 ± 0.002@@@	0.032 ± 0.002@	0.032 ± 0.002^{ns}
4.11 (*M. whitei*)	0.032 ± 0.001@	0.033 ± 0.001@	0.024 ± 0.003@@
8.22 (*M. whitei*)	0.030 ± 0.004@@	0.028 ± 0.001@@	0.029 ± 0.002^{ns}
2.05(*M. whitei*)+1 (Testosterone)	0.055 ± 0.001***	0.054 ± 0.005^{ns}	0.054±0.002***.@@@
4.11(*M. whitei*)+1 (Testosterone)	0.036 ± 0.002*	0.057 ± 0.006^{ns}	0.040 ± 0.002*
8.22(*M. whitei*)+1 (Testosterone)	0.044 ± 0.001***	0.040 ± 0.003^{ns}	0.045 ± 0.002***

4.3. *IN VITRO* EXPERIMENTS

4.3.1. EFFECT OF *M. WHITEI* ON ISOLATED RAT VAS DEFERENS

Noradrenaline caused contractions of isolated vas deferens of rats. Responses were minimal (Fig 1). Graded dose responses were obtained, with increasing concentration of noradrenaline; thus indicating dose- related contractions (Fig 1).

Propranolol which is a non-selective β–receptor blocking agent diminished the effect of noradrenaline on the tissue when noradrenaline was administered its presence. This effect is shown from the EC_{50} values in Table 13. EC_{50} values are used to evaluate the potency of a drug. The EC_{50} values in Table 13 confirm similar receptor activity between noradrenaline and propranolol and can be used to explain the dose related receptor block caused by the antagonism of propranolol. The higher the EC_{50} value the lower the potency of noradrenaline dose level due to receptor blocks by propranolol, the antagonist. Administration of calculated doses of *M. whitei* on the isolated vas deferens showed no responses, neither contractions nor relaxations. In the presence of *M. whitei*, there was no competitive inhibition of noradrenaline on the tissue, indicated by an EC_{50} of zero (Table. 13)

Table 13 EC_{50} values of dose-response curves for Noradrenaline (μg/ml) and *M.whitei* (mg/ml) on isolated Rat vas deferens, and Noradrenaline in the presence of Propranolol

DRUGS	EC_{50}
Noradrenaline	1.9788
M. whitei	-
Noradrenaline + Propranolol ($0.33x10^{-7}$M)	3.3742
Noradrenaline + Propronolol ($0.5x10^{-7}$M)	11.4376

4.3.2. EFFECT OF *M. WHITEI* ON ISOLATED RAT UTERUS.

Adrenaline is a standard drug, which is known as a smooth muscle relaxant. Administration of adrenaline to an isolated rat uterus yielded graded responses as shown in Fig 2. In trying to find the activity of *Mondia whitei* on the uterus, on its administration, graded dose responses were obtained. There were dose-related contractions followed by relaxations when the extract was applied to the rat uterus preparation. Increasing concentration of *M. whitei,* increased responses, and the dose-related relaxations of *M.whitei* on the isolated rat uterus preparation is as illustrated in Fig 3.

On simultaneous administration of propranolol, a β-adrenergic receptor antagonist with adrenaline, there was a competitive inhibition of the dose responses of adrenaline on the isolated rat uterus. In other words increasing concentrations of propranolol resulted in dose related inhibition of responses caused by adrenaline as shown in Fig 2. The EC_{50} values further show this inhibition activity as shown in these curves and the confirmation of the same receptor specificity for adrenaline and propranolol can be seen in Table 14.

There was a non-competitive inhibition of the dose responses of *Mondia whitei* on the isolated rat uterus preparation when the extract was administered in the presence of propranolol (a β-adrenergic receptor antagonist), thus as the concentration of propranolol was increased, there was a flattening out of these curves with increasing antagonist dose (Fig 3).

Table 14 EC_{50} values of dose-response curves for Adrenaline (μg/ml) and *M. whitei* (mg/ml) extract on isolated Rat uterus, and these in the presence of Propranolol

DRUGS	EC_{50}
Adrenaline	0.307
M. whitei	12.948
Adrenaline + Propranolol ($0.33x10^{-7}$ M)	-
Adrenaline + Propranolol ($0.5x10^{-7}$ M)	0.435
Adrenaline + Propronolol ($1.0x10^{-7}$ M)	1.753
M. whitei + Propranolol ($0.33x10^{-7}$ M)	38.612
M. whitei + Propranolol ($0.5x10^{-7}$ M)	75.268
M. whitei + Propranolol ($1.0x10^{-7}$ M)	1259.649

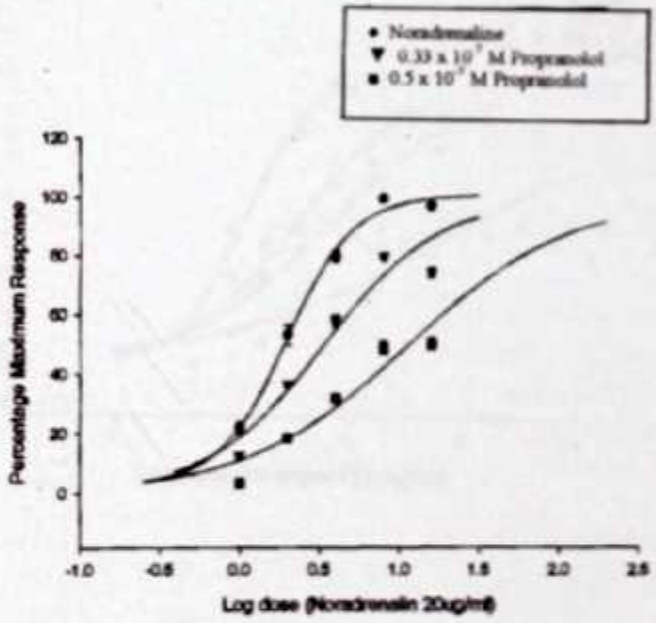


Figure 1 The effect of propranolol on the dose-response curve of Noradrenaline on the isolated rat vas deferens preparation. Administration of this standard drug was made in the presence of Propranolol at different concentration, $0.33x10^{-7}$ M and $0.5x\ 10^{-7}$M.

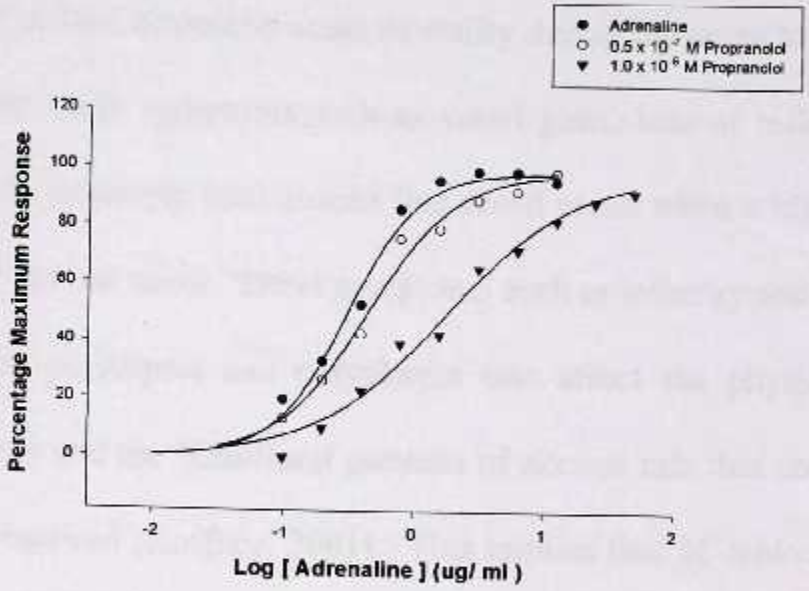


Figure 2 The effect of Propranolol on the cumulative dose-response of Adrenaline on an isolated rat uterus preparation. Administration of this standard drug was made in the presence of Propranolol at different concentrations, $0.5x10^{-7}$M and $1.0x10^{-7}$M

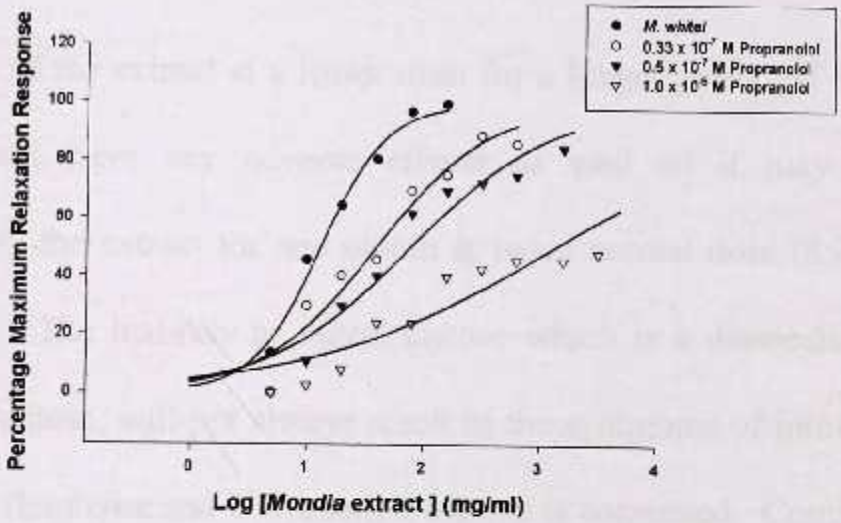


Figure 3 The effect of Propranolol on the cumulative dose-response of *Mondia whitei* (relaxation) on an isolated rat uterus preparation. *M. whitei* was administered in the presence of different concentrations of propranolol ($0.33x10^{-7}$M, $0.5x10^{-7}$M and 10^{-6}M)

CHAPTER 5

5.0 DISCUSSION

The extract of *M. whitei* caused no acute mortality during the acute toxicity tests. Certain acute drug related toxic symptoms such as crawl gaits, loss of reflexes (pupillary and righting), sedation, anorexia, convulsions that could result when a high dose of a drug is taken all at once did not occur. Other symptoms such as lethargy and fatigue, respiratory distress, polyuria, polydipsia and polyphagia that affect the physical well-being, the autonomic reflexes and the behavioral patterns of normal rats that could finally result in death were not observed (Koffuor, 2001). This implies that *M. whitei* even at dose level of 1052.16 mg/kg body weight which is equivalent to 256 times the normal dose level of 4.11 mg/kg may be safe for intake.

Administration of the extract at a lower dose for a longer period of 1 month (sub-acute toxicity) did not have any adverse effects as well so it may be assumed that administration of the extract for one month at twice normal dose (8.22 mg/kg per body weight) is safe. The inability to digest lactose which is a diasaccharide consisting of glucose and galactose, will not always result in the symptoms of intolerance (abdominal pains, bloating, flatulence and diarrhoea) if lactose is consumed. Continuous ingestion of large doses of lactose increases the expression and activity of intestinal lactose, at least in rats (Peuhkuri *et al.,* 2000). So due to the effect of the possible sugars (glucose) that are said to be found in *Mondia whitei* roots, faeces passed from rats administered with the 8.22 mg/kg *M. whitei* possibly could be responsible for the soft nature of the faeces. In the present study, no alterations were found in some soft tissues when the histological

profiles of the liver and kidneys of the rats treated sub-acutely were examined. These organs are very important internal organs whose functions are essential in maintaining life in living systems. The liver undertakes the synthesis of plasma proteins, metabolism of amino acids, carbohydrates, lipids, hormones and drugs as well as the removal of antigens absorbed from the gastrointestinal tract (Bell *et al.*, 1980). If any of these functions are impaired due to partial or total destruction of the liver hepatocytes, the defects associated with this can lead to death (Koffuor, 2001). It was very important to determine the effect of *M. whitei* extract on the liver hepatocytes, since the drug even though administered orally reaches the liver. Acute and chronic oral toxicity tests done by Shah and others (1998) with *Cinnamomum zeylanicum* showed that the oral administration of the ethanolic extract caused a reduction in liver weight and increase in haemoglobin levels, whilst *Piper longum L.* fruits caused weight increase in liver and spleen.

The kidney which is involved in osmoregulatory processes, if the nephrons or its constituents are affected by the prolonged intake of the extract, will offset the osmotic balance of the body and also result in accumulation of waste material, which are toxic to the body, resulting in death (Koffuor, 2001). It was reported that some drugs can damage the kidney therefore upon prolonged periods of use, nephrotoxicity could occur (Wilson *et al.*, 1975). Two photomicrographs of the kidneys showed some abnormalities, one with swollen cortical cells (2.05 mg/kg *M. whitei*-treated rat) and the other with calcified deposits (control rat). Since it was not possible to make a prior inspection of these kidneys and also the fact that the other animals administered with the same doses showed

nothing of that nature, it can be assumed that these two rats had developed these features due to other causes and not from the administration of the extract. From both statistical and histological evidence of the glomerular numbers and sizes, the *Mondia* root extract at the highest dose level of 8.22 mg/kg body weight (equivalent to twice the normal human folkloric dose level known to be used in Ghana) did not show any significant effect ($P \geq 0.05$). Furthermore there was no evidence of hypertrophy, dystrophy or atrophy of cells or any major other cellular disturbances. Significant size increase ($P \leq 0.05$) of glomerulus as a result of the 4.11 mg/kg treatment of *M. whitei* may have been subtle not to have caused clinical or laboratory changes in the short term. The observation cannot be readily explained except to speculate that the normal dose is stimulatory to cause the increase in sizes of the glomeruli. ½ the normal dose is a sub-threshold dose while 2x the normal dose is inhibitory. Based on the evaluation of the liver and kidneys alone, it could be assumed *M. whitei* is safe to consume.

During chronic toxicity tests, the extract caused no significant chronic mortality when compared to the controls except for some 2 rats one each from the normal (4.11 mg/kg) and twice the normal doses (8.22 mg/kg) of *M. whitei*-treated rats that died. This could mean the two deaths that occurred could have been as a result of some inherent infection rather than as a result of the administration of the extract since all other rats were reported to be physically healthy and increasing in body weight. A head tilt observed with circular movements are mostly clinical signs exhibited by rats suffering from a bacterial disease caused by *Mycoplasma pulmonis* transmitted either via aerosol-infected secretions or via uterine infection (Pass and Freeth, 1993). These deaths could have resulted from the

head tilt that caused abnormal movements and inactivity of the rats. A general weight loss observed was due to their inability to feed or eat well due to the head tilt. Three rats that had their hair bitten off (two from the 2.05 mg/kg *M. whitei*-treated and one from the control rats) suffered from a condition known as barbering. Barbering which is caused by fights among rats that co-exist in a single cage, is a common non-infectious condition. Barbering therefore cannot be the result of a toxic effect of *M.whitei.* Hair loss could also be due to mites, the fur mite (*Radfordia ensifera*) of the ear mange (*Notoedres muris*) which is called pruritis (Pass *et al.*, 1993).

All concentrations of the extract did not distort the histological profiles of the liver and the kidney even after 3 months of exposure. Thus, the one rat that was administered with the normal dose and showed the fluid packed swollen cortical cells in the kidney profile may have been from causes prior to the experimental time (Plate 3F). No hypertrophy, dystrophy and atrophy occurred in the tissues of the liver and kidneys since there were no significant differences in the shapes, numbers and sizes in cells of the organs of the drug-treated rats when compared to the untreated histologically, so the results of this present toxicity studies provide basic information about the possible safe use of *M. whitei* folklorically in Ghana.

Noradrenaline induces contractions in rat vas deferens and this effect is known to be brought about by interaction with specific α_1. adrenergic receptors in the smooth muscle cells. From experiments performed, *M. whitei* did not cause any such contraction on vas deferens as noradrenaline would. This could indicate that *M. whitei* possibly acts on β_2-

adrenoceptors. *M. whitei* could also possibly be act on the noradrenergic nerves of which the transmitter is unknown, since previous studies have suggested the existence of a non-adrenergic component which is prominent in the prostatic half of the vas deferens Kitchen, (1984b) and also Sneddon and Machaly (1992) from their research reported that noradrenaline was significantly potent in producing contraction in the epididymal segments of the vas deferens muscle than in prostatic segments. Literature clearly shows that the contractile effect of noradrenaline is solely through α_1-adrenergic receptors (Minnerman *et al.*, 1983). Since β_2-adrenoceptors do appear to be present in the mouse vas deferens, whose activity initiated by isoprenaline and salbutamol is blocked by propranolol, it can be said that contraction caused by noradrenaline could be due to these β_2-adrenoceptors (Kitchen, 1984b).

Experiments suggest that the potencies of agonists in activating α_1-adrenergic receptors in rat vas deferens agree well with their potencies in binding to the receptors. The greater potency of agonists in causing a contraction may be due to spare receptors in the tissue (Minneman and Abel, 1984). There could be more spare α_1 sites for noradrenaline than *M. whitei* to act on, if it should act on α_1-sites to induce contraction and also *M. whitei* being less selective for the α_1-adrenoceptor subtype.

As reported by Medina and others in 2000, vas deferens motility which can either be initiated through the inhibition of Ca^{2+} entry and an effect on adrenergic receptors or just by the inhibition of Ca^{2+} entry could explain the effect in the experiments performed

Propranolol, which is a non-selective β adrenergic blocking agent, could have inhibited contraction by noradrenaline through inhibition of Ca^{2+} entry with no effect on the adrenergic receptors as reported on setraline and fluoxitine inhibition of motility elicited by noradrenaline in vas deferens (Medina *et al.*, 2000). *M. whitei* can be probably said not to inhibit Ca^{2+} entry therefore results in not inhibiting the action of noradrenaline on the vas deferens and also therefore not affecting the motility of vas deferens when the extract was administered alone. Two subtypes of α_1-adrenoceptors have been reported to cause contractile responses through different molecular mechanisms, of which one subtype stimulates inositol phosphate formation and causes contractions which require the influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels (*Han et al.*, 1987). *M. whitei* may also not have stimulated any α_1-receptor subtype to in turn stimulate inositol phosphate formation to cause any contractions of the vas deferens when administered alone.

In this study, *M. whitei* was shown to reduce the sponetaneous contractile movements of rat uterine smooth muscle. This inhibition was concentration dependent. But β_2-receptor stimulation is known to mediate relaxation of vascular and uterine smooth muscle (Westfall, 1990). *M. whitei* could probably be said to have a β-adrenergic activity and no α -adrenergic activity. Activity of *M. whitei* in the presence of different concentrations of propranolol confirmed a non-competitive inhibition that could either be reversible or irreversible.

The uterus is innervated by sympathetic nerves and possibly a parasympathetic supply which runs to the wall of the tissue. In the rat uterus, β-receptors remain constant, but α -receptors appear under the action of oestrogen. The β-adrenergic receptor responses predominate but it sometimes preceeded by a transient α -adrenergic receptor mediated contraction for mixed α- and β-agonist (Kitchen, 1984a). Since the isolated uterus has no inherent tone, relaxation can only be observed well by physiological antagonism to the contractile responses to drugs such as acetylcholine and β-receptor-mediated relaxation of the uterus preparation can be blocked with β-receptor antagonists such as propranolol (Kitchen, 1984a).

Administration of the *M. whitei* extract resulted in the same relaxing response produced by adrenaline with increasing concentrations. It was reported that noradrenaline and adrenaline both decrease uterine contractile activity and that decrease in adrenaline concentrations causes increased uterine activity (Segal *et al.*, 1998). This could be indicative of the fact that *M. whitei* could act on the same receptors that adrenaline acts on in the uterine tissue. The comparison between the effects of *M. whitei* extract (in the presence of propranolol) and that of adrenaline (in the presence of propranolol) confirms that *M. whitei* has β-adrenoceptor activity.

Aqel and others, in 1991 (quoting Van Breemen *et al.*, 1982) stated that contraction of the smooth muscle is dependent upon an increase in the concentration of cytoplasmic-free Ca^{2+} which activates the contractile elements. The source of activator Ca^{2+} may be extracellular or intracellular. The relative contribution of Ca^{2+} from these two sources to

contraction however, largely depends upon the type of smooth muscle tissue and the contractile agent in question, the concentration of the agent and the component of the contractile response (whether phasic or tonic) being examined. The spontaneous movement of uterine smooth muscle is regulated by cycles of depolarization and repolarization. Aqel *et al.*, (1991) (quoting the work of Boltin, 1979), also reported that action potentials appear at the height of depolarization and constitute a rapid influx of Ca^{2+} via voltage dependent Ca^{2+}-channels (VDCs). Since *M. whitei* extract inhibited the spontaneous movements of the rat uterine smooth muscle, it may interfere with either the depolarization process or with Ca^{2+} influx through VDCs. If *M. whitei* could have a deplolarizing effect on the uterine muscle producing the contractile effect as *Ferili sinaica* root extract has been proposed to do, then it can be assumed that the *M. whitei* root extract may not only probably have a β-receptor adrenergic activity but may block the Ca^{2+} influx via the Ca^{2+} channels so that the full contractile effect of the uterine smooth muscle is reduced or the full relaxing effect of the extract is induced. As reported by Burgos and others (2001) that *Andrographis paniculata* completely blocked the contractile response of acetylcholine by blocking voltage calcium channels, relaxation by *M.whitei* can be said to be due to the effect on the voltage calcium channels of the uterine muscle.

The observation that administration of root extracts of *M. whitei* significantly increased body weight may be due to the extracts ability to elaborate high growth hormone which is very effective during the first 2 weeks of administration (Table 5). Choudhary *et al.*, (1991) indicated the influence of the growth hormone through observations they made

when working with leaf extracts of *Alstonia scholaris, Cleistanthus collinus and Terminalia bellirica* was at a dose of 100 mg/kg/day for 21 days. This effect was more pronounced when extract alone was administered.

Malini and Vanithakumari (1991) quoting the work of Steinberger and Steinberger (1972) stated that development and functions of testes and accessory sex organs are closely dependent on testosterone and gonadotrophic hormones. The testes secrets large amounts of androgens, principally testosterone and small amounts of estrogens. Androgens are also secreted from the adrenal cortex (Ganon, 1995). Exogenous administration of androgens to normal males inhibit the release of pituitary gonadotrophins as a consequence of which a decrease in the testicular production of testosterone occurs, in turn spermatogenesis is also reduced (Schwartz and Miller, 1990). Tuffour (2000) (reporting on the work of Thomas and Mawhinney, 1973) indicated that any agent that is capable of antagonizing or interfering with the actions of testosterone or its close derivatives may be referred to as an anti-androgen. The anti-androgen may suppress the metabolic actions of male sex hormones, or it may interfere with the uptake, sub-cellular distribution, binding of androgens. Still, the anti-androgen does not necessarily have to exert its antagonistic action directly upon an androgen target cell. Rather it may suppress pituitary gonadotrophin secretions and thereby indirectly interfere with androgenic responses. The chronic administration of such compounds to male rats leads to a castration-like action upon androgen target organs. Malini and Vanithakumari (1991), (quoting Means, 1986) reported that the weight of testes is known to be an index of FSH. Again, Malini and Vanithakumari, 1991 (quoting Saunders, 1958) said it has

been confirmed that both steroidal and non-steroidal agents inhibit pituitary gonadotrophins either by acting directly on the pituitary or through the hypothalamo-hypophyseal axis as reported by Bogdanove, in 1963 (Malini and Vanithakumari, 1991).

M. whitei does not affect testes weight *per se*, because when administered alone did not cause any significant changes. Increases that occurred when the ethanolic extract at dose of 2.05 and 4.11 mg/kg were administered along side with testosterone when compared to testosterone administration alone could be due to some possible synergistic effect of both *M. whitei* and testosterone administered at low doses. *M. whitei* appears to have no anti-androgenic activity. *M. whitei* at dose levels used did not show any antifertility effects as the ethanolic extract of *Colebroo-kia oppositifolia* caused a reduction in the testis and epididymal weight (Nivsarkar *et al.*, 2002).

Watcho and others in 2001 reported that a chronic administration of *Mondia whitei L.* root back extract (400 mg/kg/day) for 55 days even though did not cause any change in testes weight, epididymis and ventral prostate weights. But this same dose caused testicular lesions resulting in the cessation of spermatogenesis, degenerative changes in the seminiferous tubules and epididymis. The treatment resulted in a partial antifertility, but allowing a recovery period, normal spermatogenesis and fertility resumed, suggesting reversible antispermatogenic and antifertility effects of the plant. Histological studies did not show any such testicular lesions and degenerative changes, therefore an intake of *Mondia whitei* even at a dose that is twice (8.22 mg/kg) as recommended to be taken by users in Ghana, as indicated by the Center for Scientific Research into Plant Medicine, at

Akwapim Mampong did not cause any non-reversible or reversible antifertility effects, confirming the absence of any antiandrogenic and antispermatogenic nature of the extract. As reported by Golalipour and others (2003), that *Achillea santolina* at a dose of 300 mg/kg administered for 20 days showed not only some alterations in the spermatogenesis process but a decrease in testes weight as well which did not occur in experiments with *Achillea millefolium*. They reported that the differences in results of both studies might be either due to the use of different varieties of *Achillea* or a higher concentration of *A. santolina*. The differences in this study with *M. whitei* (8.22 mg/kg for 3 weeks) and *M. whitei L* (400 mg/kg/mg for 55 days) could be due to the different dose levels of extract and time lapses for both experiments. This could infer that its temporary effect could be only dose and time dependent.

Hiremath *et al.*, (1997) (quoting the work of Mann and others, 1981) reported that the antiandrogenic action of an ethanolic extract of *Striga orbanchidioides* itself explained the histological changes that were observed in the testes of rats from the experiments that were performed. A significant reduction in the spermatogenic elements, and the absence of mitotic/meiotic cell division in the seminiferous tubules are due to the inhibition of androgenic activity, which is said to be essential for spermatogenesis. Administration of *M. whitei* at the doses that were used in this study did not cause a reduction of spermatogenic elements and therefore did not seem to inhibit pituitary gonadotrophins or androgens.

Increase in weight of epididymis induced by administration of the ethanolic extract of *M. whitei* and testosterone when compared to controls and testosterone administered controls suggests no antiandrogenic activity of *M. whitei*. The epididymis is dependent on androgens (Hiremath *et al.*, 1997) so its increase in weight observed for the combined treatment of half the dose of the *Mondia* extract and testosterone could possibly indicate androgenic activity of the extract or just the effect of testosterone on the organ, since the drug alone did not cause any significant changes in epididymal weight (Table 7). Herbal extracts of *Momordica charantia* was said to have antispermatogenic but androgenic activity due to increases in accessory organ weights such as the epididymis (Naseem *et al.*, 1998). For dose levels of *M. whitei* used, it cannot be said to be antiandrogenic.

As reported in a paper by Malini and Vanithakumari in 1991, certain drugs or substances could cause decreases in sperm count and accessory organ weight due to their intrinsic oestrogenic or anti-androgenic activities, (it was well stated that these responses vary according to age, species, hormonal status, gland type and types of tissues within a single gland). The oestrogenic hormone which could inhibit male gland secretion at larger doses, could have a stimulating effect at smaller doses. *M. whitei* reduced weights of seminal vesicles after a week of treatment when compared to the testosterone treated (Table 8), indicating an inhibitory effect. The simultaneous administration of ethanolic *M. whitei* extract and testosterone induced reduction in the weights of the seminal vesicles compared to the testosterone treated within the same week confirming its inhibitory effect in this gland type, indicating possible androgenic activity for the administration time of a week. Results after 3 weeks of the administration of half the

normal dose (2.05 mg/kg) combined with testosterone actually had a stimulatory effect. This may well explain possible stimulatory influence of *M. whitei* on growth of accessory sex organs such as seminal vesicles and epididymis, at a low dose level co-jointed with testosterone. This effect can be said to be time dependent though.

Androgenic activity of 2.05 mg/kg dose level of the extract combined with testosterone again in the last week of treatment can be assumed again. Malini and Vanithakumari (1991) (quoting Steinerger and Steinberger in 1972) reported that increase in gonadotropins and testosterone plays a vital role in the growth and development of accessory sex organs. This result by the end of the third week could also be said to be due to increase of gonadotropins through the effect of testosterone. Even though histologically *M. whitei* treated seminal vesicles showed fluid filled cavities or lumina, this was not enough to show any significant increase in organ weight when compared to the controls, though histologically, it had well formed lumina and epithelial membranes compared to the controls (Plate 23B).

Antiandrogens are reported to prevent androgens from maintaining their cellular activities (as reported by Brandes in 1974) (Malini *et a*l., 1991). The extract alone showing weight increase of the ventral prostate in 1st week, and also increases with combined treatments, which occured mainly in the last week, could possibly indicate androgenic activity of development of the ventral prostate (Table 10). These occurrences after 3 weeks of combined administration especially with the half the normal dose plus testosterone could possibly confirm that *M. whitei* at low dose level combined with testosterone has a

stimulatory effect that is time dependant as in other accessory organs including the testosterone treated. Hiremath and others in 1997 stated that some flavonoids possess oestrogenic activity that inhibits the action of 5α reductase in the prostate organ, which is essential for its growth. Hiremath *et al.*, (1997) (quoting Bhargava, 1989) further reported that flavonoids produce antiandrogenic activity and affect male fertility. The observed increases of the organ weight may be due to the androgenic nature of *M. whitei*. An aqueous extract of *Myrica rubra*, showed *in-vitro* testosterone 5 alpha-reductase inhibitory activity in prostate and *in-vivo* antiandrogenic activity due to its constituent myricetin, a flavonoid (Matsuda *et al.*, 2001). This could possibly indicate that *M. whitei* root extract does not contain any such flavonoids. The ventral prostate glands of the extract-treated rats (Table 11) during the 1st week of treatment showing very significant increases in epithelial cells with lumina and septae looking normal confirms lack of antiandrogenic activity of the extract. The wider widths of secretory columnar epithelium observed could mean more secretion of fluid as observed in most of the photomicrographs (Plate 10F).

Significant reduction in weight of vas deferens of extract-treated rats compared to the testosterone treated occurs almost throughout all the administration period. Stimulatory effects or increases with combined treatments especially in the third week, confirms that low doses combined with testosterone induces a stimulatory effect as in the accessory organs studied.

CHAPTER 6

6.0 SUMMARY OF MAIN FINDINGS

1. *M. whitei* is well tolerated up to a dose of 1052.16 mg/kg body weight which is equivalent to 256x the normal dose level of 4.11 mg/kg body weight when administered orally for the toxicity tests. The results of the present toxicity studies provide basic information about the possible safe use of this herbal extract and the necessary measures to be introduced during the preclinical testing of the *M. whitei* ethanolic extract in the future.

2. After a series of well defined scientific experiments, some conclusions can be drawn. It can be concluded that *M. whitei* at the folkloric dose has no anti-androgenic effect on the male reproductive processes or fertility, at dose levels that were used in this experimental set up. No testicular lesions and damage to testis were seen but *M. whitei* at the lowest dose level combined with testosterone for the seminal vesicles and ventral prostate weights, a possible stimulatory influence of *M. whitei* which is actually time dependent.

3. *M. whitei* did not inhibit the vas deferens smooth muscle contraction induced by noradrenaline stimulation. The extract can be said not to have any α-adrenergic antagonism on effects of noradrenaline on vas deferens as propronolol which has β-adrenergic antagonism, and it does not affect Ca^{2+} entry, therefore not affecting vas deferens motility.

4. *M. whitei* inhibited the smooth muscle contraction in the uterus. This ethanolic extract can be said to have a purely β-receptor adrenergic activity as adrenaline and a possible inhibition of Ca^{2+} influx through VDCs which is vital for contraction of the uterine smooth muscle. Due to *M. whitei*'s relaxing activity or effect, it could be used to possibly maintain pregnancy and prevent some types of spontaneous abortions in pregnant women arising from uterine contraction.

6.1 CONCLUSION AND RECOMMENDATIONS

6.1.1 CONCLUSIONS

It has been shown in this work that the alcoholic extract of *M. whitei* is not toxic to rats even up to an equivalent of 256× of the folkloric dose. *M. whitei* at twice the folkloric dose had no visible effect on the spermatogenesis, the testicular tissue nor the accessory organs. This same dose had no antiandrogenic effect nor did it affect fertility.

M. whitei has a relaxing activity on the uterine smooth muscle preparations, but has no effect on the smooth muscle of the vas deferens.

6.1.2 RECOMMENDATIONS

According to the fertility studies performed by Watcho and others in 2001 with *M. whitei L.*, higher doses and longer periods of administration were used and it was reported to produce a reversible anti-fertility effect, as compared to this research. Further experiments with much higher doses used over variable periods should be done to confirm the reversible anti-fertility nature of *M. whitei* in male albino rats since the

highest dose of 1056.16 mg/kg was used only for the acute toxicity tests.. The effects of these high doses on soft tissues should be examined. Parameters such as the number of leydig cells, the diameters of their nuclei and diameters of testes and seminiferous tubules should be measured to evaluate whether some micro parameters could have been overlooked in the present work.

The acclaimed folkloric aphrodisiac properties of *M. whitei* should be investigated through experiments in which number of penile erections, homosexual mounting, copulatory behaviour and orientation activity in rats should be compared to controls.

A further study is also necessary to isolate active principles from *M. whitei* roots, determine their mechanisms of action on all accessory organs and to check the possibility of any hormonal effects on any of these organs. *M. whitei* plant, after being subjected to chemical extraction, isolation procedures should be done in order to characterize the pharmacologically active principle(s) and their mechanisms of action that result in its effects on uterine activity. Advanced pharmacological investigations of the active principle(s) can be undertaken.

Experiments to determine a possible inhibition of Ca^{2+} influx through VDC's which is vital for contraction of the uterine smooth muscle can be done.

Effects of *M. whitei* on luteal activity, implantation and litter size, studies into its possible maintenance of pregnancy are very necessary.

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