EFFECT OF BI-HERBAL FORMULATION OF *Carica papaya and Vernonia amygdalina* ON THE REPRODUCTIVE SYSTEM OF MALE ALBINO WISTAR RATS

BY

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DEPARTMENT OF SCIENCE LABORATORY SCIENCE

FACULTY OF LIFE SCIENCE

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2022.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc. HONOURS) DEGREE IN SCIENCE LABORATORY

(PHYSIOLOGY AND PHARMACOLOGY)

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CERTIFICATION

This is to certify that this research work was carried out by Destiny Orobosa IZEKOR with Matriculation number LSC1608034 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to the Almighty God for His divine guidance and to my parents, late. Mr. Joshua Izekor of blessed memory and my mom, Mrs. Betty Izekor for their love and support all through the course of my study.

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I am most grateful to God Almighty for the gift of life, divine health and for the strength to finish strong.

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May God richly bless you all!!

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ABSTRACT

This study evaluated the effect of N-hexane leaf extract of bi-herbal formulation of *Carica papaya* and *Vernonia Amygdalina* on the reproductive system of male reproductive system. A total of 25 Wistar rats were used in total for the conduction of the experiment. The animal was distributed into six groups comprising of five rats and fed with grower feed for a period of 28 days. At the end of the experiment, the animals were sacrificed and subjected to laparotomy after which the testes, prostate, seminal vesicles and right epididymis were isolated, blood and sperm samples were also collected for hormonal assay, sperm analysis and histopathology analysis. The study revealed no mortality, loss of cognitive/ loss of agility or any physical morphology associated with toxicity in any of the treatment group. A significant reduction in weight index was recorded in the extract treated groups. Previous studies done on this plant showed *Vernonia amygdalina* and *Carica papaya* exhibiting weight loss properties individually, hence, the reduction can be attributed to the rich mileu of phytochemicals in both plants. Another mechanism suggested for the weight loss, is the interference of pancreatic lipase by the extract. This causes inhibiting or delay in the digestion and absorption which consequently result in a reduction in body weight. However, there was no significant difference in the organ weight and relative organ weight of the extract when compared to that of the control group. Total sperm count was significantly lowered in all treatment groups. Decrease in the progressive motility and normal cell counts were respectively recorded. The result of the study shows that the bi-herbal

formulation of *Carica papaya* and *Vernonia amygdalina* had a negative effect on the sperm and reproductive parameters which is crucial for fertility, thus the extract poses as a likely candidate for contraception.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Plants have been used since ancient times for a variety of functions within human as well as animal existence, ranging from being used as a source of nourishment to the exploitation of their therapeutic composition, employed for the prevention coupled with management of a plethora of mild and severe disease. The therapeutic activities exhibited by these medicinal plants are as a result of their bioactive constituents which includes: flavonoid, saponins, alkaloids as well as many others (Bonsi *et al*., 1995). In the scientific world, these medicinal plants are referred to as "herbal medicine" or "phytomedicine". They are herbal preparation which have plant or components as active ingredient. These components include seeds, fruit, roots, leaves, bark or flowers. Several of these plants' components have long been utilized in traditional medicine. Traditional medicine is a diverse array of health practices, knowledge coupled with belief which includes physical procedures coupled with exercises that are derived from plants, animals or minerals which are used either alone or in combination to preserve health. People from all around the world have developed unique indigenous healing techniques which have historically fulfilled their community health care need (Igile *et al*., 1995). In both advanced as well as emerging economies, there has been a major growth in public acceptance in addition to interest in natural therapy over the last decade. These herbal formulations are used for the symptomatic relief and treatment of illnesses due to their accessibility, affordability coupled with immunological activities against a wide spectrum of chronic diseases (Shakya, 2016). Some of these plants whose extracts has been employed as a treatment for diverse diseases around the world includes: *Vernonia amygdalina as well as Carica Papaya*.

Vernonia amygdalina is a common plant in African which is consumed as a vegetable and has significant therapeutic potential. The leaves are beneficial in the preparation of herbal medication (Ifeolu and Oyetunde, 2018). *Carica Papaya* is commonly referred to as 'pawpaw'. Different portions of the plant are used around the world to treat both human as well as veterinary conditions.

1.2 AIMS OF THE STUDY

The aim of the study is to investigate the effect of bi-herbal formulation of *vernonia Amygdalina* as well as *Carica Papaya* on the reproductive system of male albino Wistar rat.

1.3 OBJECTIVES OF THE STUDY

1. To evaluate the fertility effect of the bi-herbal formulation of *Carica Papaya* as well as *Vernonia Amygdalina* in Male albino Wistar rat
2. To determine the effect of the bi-herbal formulation of *Carica Papaya* coupled with *Vernonia Amygdalina* on the weight index of Male albino Wistar rat
3. To determine the effect of the bi-herbal formulation on sperm quality.

2.1 CHAPTER TWO

2.1.2 LITERATURE REVIEW

Herbal remedies have been employed since time memorial for the treatment of a variety of acute and chronic diseases. These plants have played a significant role in health system around the world. Numerous health conditions including pharyngitis as well as hypertension are widely treated with herbal plants. Some of these plants include the common *Vernonia amygdalina* and *Carica Papaya*. This plant contains an abundance of chemical compounds, and have demonstrated a number of medicinal capabilities including antioxidant, neuro-protective and anti-inflammatory effect.

2.1.3 Taxonomic hierarchy of *Carica papaya*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Caricaeae

Genus: Carica

Species: Papaya L.

Kaliyaperumal *et al*., 2014.

PLATE 1: DIAGRAM OF *CARICA PAPAYA*

(Malik *et al*., 2013)

2.1.4 Description of *Carica papaya*

Carica papaya popularly known as pawpaw is a single-stemmed perennial plant belonging to the genus Carica of the family Caricaceae. It is a tree-like herbaceous plant with a rapid growth rate usually growing up to height of 20m; producing fruits and reaching maturity within a year (Vijay *et al*., 2014). It has large palmately- lobed leaves (measuring 50 - 70cm in diameter) that are spirally arrange and clustered at the developing tip of the trunk (Singh *et al*., 2020). Being a triecious plant, the type of inflorescence produced are usually determined by the sex of the plant. The flowers are produced along the trunk from the leaf axil. The male species can be distinguished from other species by their long, many-flowered inflorescence making them very conspicuous. They usually lack pistil except for flowers at the distal terminus producing pistils intermittently. The female species have short inflorescence producing flowers much larger than the male species that are often solitary in the leaf axils. They have a functional pistil but without stamens. Hermaphrodite specie have short inflorescences having both a pistil as well as stamen. They are primarily self-pollinated (Ming *et al.,* 2006).

The papaya fruit are fleshy, oblong/pear-shaped fruits that changes from green to light/dark yellow when it ripens. At the central cavity lies its black seeds. The Unripe papaya fruit is known to contain a protease enzyme called papain (Da Silva *et al*., 2007).

2.1.5 Origin and geographical distribution of Carica Papaya

Although the provenance of *Carica Papaya* is unknown, it is presumed to be native to Tropical Mexico as well as Central America. In the tropical, coupled with the subtropical part of the world, including Nigeria, South East Asia, India as well as Hawaii, it is extensively cultivated (Jaime *et al*., 2007).

2.1.6 Phytochemicals of *Carica Papaya*

Using various screening tests, phytochemical examination of different quantities of papaya leaf extract led to the identification of natural compounds such as saponin, flavonoids as well as steroids. The analysis also disclosed the antimicrobial effect of Carica Papaya. It was observed the extract decreased the colony count of E. faecalis and Candida albicans (Snigdha, 2019). Paw-paw seeds, in addition to the pericarp has been reported to contain benzyl glucosinolate coupled with isothiocyanate (Nakamura *et al*., 2007). The hexane, ethyl acetate in addition to the ethanol papaya leaf extract contained phenolic compounds as well as some carbohydrate. These phytochemicals were more prominent in the methanol extract. (Nandini *et al*., 2020). An analysis carried out to determine the biological active compounds present within the unripe fruit aqueous extract, the result revealed the presence of numerous phytochemicals. Hexadecanoic acid was the primary phytochemicals while the minor phytochemical compounds ranged from 0.78 to 0.58 % (Ezekwe and Chikezie, 2017). Ayoola and Adeyeye, (2010) study revealed different composition of phytochemicals, vitamins and minerals in green, yellow and brown papaya leaves. Post-analytical result revealed 0.94 thiamine in green leaves, while the yellow and brown leaves contained 0.04 and 0.06 thiamine respectively. The green leaves contained 0.31 riboflavin content, 0.04 in yellow leaves coupled with 0.06 within brown leaves. Green leaves were also observed to have contained the highest mineral values, having 8612.50 (mg/kg) of Ca, 17.82.0(Na), 2889.00(k) and Mn (9.50) (Ayoole and Adeyeye, 2010)

2.1.7 Ethnological importance of *Carica papaya*

The milky papaya latex contains an enzyme known as papain, which has several noteworthy applications in both the industrial as well as the medical sector. It is applied in the textile sector to soften wool in addition to degumming silk. The food biotechnology sector uses it to manufacture chewing gums, dried pulses and beans as well as to tenderize meat. Additionally, papain has been used as an ingredient in making soaps, toothpaste coupled with skin care product (Cragg *et al*., 1997). It is used in Jamaica as topical ulcer dressing. In Pakistan, India as well as Sri lanka, traditional healers employ the green fruit as a contraceptive. In Nigeria, it is used to treat conditions like jaundice in addition to diabetes mellitus (Dhanamani *et al*., 2011). In India, the leaves are used to alleviate asthma and vitamin B1 deficiency (Beriberi). The latex when applied topically promote healing of burn-related wounds (Giordani *et al*., 1997).

2.1.8 Pharmacological activities of *Carica papaya*

Extensive research on *Carica papaya* has revealed that papaya plants parts such as the leaves, seeds, stem displayed numerous pharmacological activities; they include**:**

2.1.9 Anti-inflammatory effect: The anti-inflammatory properties of paw-paw has been widely studied using a number of in vivo experiments. Ethanolic leaf extract of *Carica papaya* was found to lessen inflammation within arthritic rats (Owoyele *et al*., 2008). Pawpaw seed extract was also observed to have caused a reduction of inflammation inside wistar albino rats when eggs albumin-induced inflammation was evident (Amazu *et al*., 2010). Similar results were obtained within rats with formalin coupled with carrageenan-induced pedal edema using a concentration of 400mg/kg (Ahmed and Ramabhimalah, 2012). In an in vitro study, the aqueous extract of papaya seeds significantly decreased nitric oxide radical by 69.4%. At 150g/dl, the aqueous extract inhibited enzymatic activities as well as stabilized erythrocyte level within the membrane (Wijesooriya et al., 2019).

2.1.10 Anti-diabetic effect: A reduction of postprandial glucose levels was observed after administration of ethyl acetate extract to diabetic rats. Ethyl acetate extract had antioxidant coupled with enzyme inhibitory effects in vitro, along with effects on glycosidase as well as amylase. Hexane was seen to display higher enzymatic inhibitory activities (Agada *et al*., 2020). At a concentration of 50g/ml, fermented papaya preparation (FPP) exhibited protective effect against diabetes sequels such as development of atherosclerosis plaque, elevated SOD level as well as ameliorated lipid peroxidation. Additionally improving the fluidity of the platelet membrane within diabetic patients coupled with preventing platelet dysfunction caused by chronic hyperglycemic (Raffaelli *et al*., 2015).

2.1.11 Neuroprotective effect: Fermented papaya preparation showed neuroprotective effect by reducing ROS production within APPsw cells (Zhang *et al*., 2006). When administered to Alzheimer disease (AD) patients, FPP substantially decreased the levels of 8-hydroxy2'-deoxyguanosine. Carica plant efficacy in easing AD was demonstrated by the fact that no neurotrophic medication was given to the study subjects during the trial period. The formation of free radicals, decreased lipid peroxidation coupled with neuronal toxicity brought on by aluminum and iron are some of the postulated mechanisms of action of FPP (Barbagallo *et al*., 2015).

2.1.12 Periodontal effect: The severity of chronic periodontitis as well as irritation was reduced considerably in test subjects who used toothpaste containing *Carica papaya* constituents (Saliasi *et al*., 2018).

2.1.13 Anti-aging effect: The ethanol leaf extract increased the formation of type 1 procollagen as well as reduced the degree of degradation within fibroblast exposed to UVB radiation in addition to exhibiting dose-dependent radical scavenging coupled with ROS suppressing (Seo *et al*., 2020).

2.1.14 Chemo-protective Effect: Carica papaya displayed this effect by significantly reducing the number of pulmonary adenomas within carcinogenic animal model. A huge margin difference was observed between the extract as well as the control group, as the incidence of cutaneous papillomagnesis was substantially lowered in the animals that received the extract. It was proposed that the flavonoid found within various Carica papaya plant parts function as effective chemopreventive agents through multi-signaling network (Pathak et al., 2014). *Carica papaya* peel extract substantially elevated glutathione while reducing MPA in addition to ROS generation. As a result, the group treated with azoxymethane averted DNA damage coupled with the development of colon cancer (Waly *et al*., 2014).

2.2 Taxonomic hierarchy of *Vernonia Amygdalina*

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledons

Order: Asterales

Family: Asteraceae

Genus: Vernonia

Species: *Vernonia amygdalina*

Owoeye *et al*., 2010

PLATE 2: DIAGRAM OF *VERNONIA AMYGDALINA*

(Lian and Scott, 2014)

2.2.1 Description of *Vernonia Amygdalina*

Vernonia amygdalina belongs to the Asteraceae family. It is also commonly known as bitter leaf as a result of its bitter taste. It is commonly used as vegetable (Igile *et al*., 1995). It goes by a variety of names in Nigeria, they include; ‘’Ewuro’’ in Yoruba, ‘’Ityuna’’ in Tiv and ‘’Onugbu’’ in Igbo (Igile *et al*., 1994). The leaves are elliptical in shape and can reach a height of 20cm (Johri and Singh, 1997)

2.2.2 Phytochemicals of *Vernonia Amygdalina*

There are numerous chemical compounds within *Vernonia Aygdalina*, some of which have been discovered to have strong biological effects. Flavonoids, saponin, alkaloids, steroids, reducing sugars, triterpenoids were found inside bitter leaf extract that was tested for phytochemicals (Aiyegoro and Okoh, 2009). The isolation of the compound Elemanolide from dichloromethane extract of bitter leaf was reported by *owoeye et al.,* 2010

2.2.3 Ethnological importance of *Vernonia amygdalina*

Vernonia amygdalina is a multipurpose plant which has plethora of traditional applications. The leaves are commonly used as vegetables in Nigerian soups, particularly the classic bitter-leaf soup. They are eaten as appetizers, while the leaves extract is used to promote digestion as well as being utilized for beer-making (Singha, 1965; Babalola and Okoh, 1996). It is consumed by the female folks of the Hausa tribe in the northern part of Nigeria with the idea that it improves their sexual appeal. It is also added to horse feed to create a fortifying medicine. (Daziel, 1937). The leaves are utilized as a quinine substitute within various African nations, particularly Nigeria, due to their well-known anti-fever properties. The young fresh leaves are used to treat high blood sugars, fevers, constipation, hypertension as well as being used as a laxative in Southern Ghana (Asante *et al*., 2016). They are also used to treat wound, hepatitis and sexually transmittable infections. The leaves are used to increase female fecundity as well as to improve lactation in nursing mothers (Adedapo *et al*., 2014). *Vernonia Amygdalina* has also been reported to have some veterinary application. It is used for the treatment of fever in poultry, cattle mastitis in addition to livestock helminthosis (Nabukenya *et al*., 2014).

2.2.4 Pharmacological activities of Vernonia Amygdalina

Diverse investigation has been conducted to explore the pharmacological characteristics of *Vernonia Amygdalina* in order to support the plant's long-standing usage as a remedy. This research has proven *Vernonia Amygdalina* to be a plant with a plethora of pharmacological activities. The pharmacological activities of *Vernonia Amygdalina* includes:

2.2.5 Anti-diabetic effect: The anti-diabetic efficacy of the flavonoid portion of bitter leaf was reported by Ebong *et al*. in 2006. The research group which received the extract was observed to have experienced a reversal in pancreatic beta cell damage. This regeneration of damaged pancreatic beta cell could be considered as a viable mechanism for *Vernonia Amygdalina* anti-hyperglycemic effect.

2.2.6 Antipyretic effect: The saponin component of *Vernonia Amygdalina* was seen to have demonstrated antipyretic effect. At a concentration of 100mg/kg and 200mg/kg, a decrease in anal temperature was observed (Johnson *et al*., 2015).

2.2.7 Anti-inflammatory effect: *Vernonia Amygdalina* significantly reduced inflammation on the right ear of research mice induced with four parts of pyridine, one part distilled, five parts of diethyl ether and 10 parts croton oil in diethyl ether (v/v) (Georgewill and Georgewill, 2010).

2.2.8 Analgesic effect: Research mice writhes caused by acetic acid were lessened by the aqueous extract of VA (100mg/kg and 200mg/kg), thus demonstrating its analgesic effects. The extract's analgesic efficacy at both doses was on par with that of the widely used medication indomethacin (Adedapo and Aremu, 2014).

2.2.9 Spermatogenic effect: The effects of *Vernonia amygdalina* extract on sperm production was described by Saalu and Akunna (2013). A better sperm quality resulted from the treatment with this extract (50mg/kg and 100mg/kg). It had the opposite effect, though, at a greater dose (200mg/kg) (Saalu and Akunna, 2013; Longe *et al*., 19830).

2.2.10 Leishmanial effect: Study revealed that the hexane and aqueous extract of Vernonia Amygdalina can decrease the occurrence of leishmaniosis infection. This effect can be attributed to its flavonoid composition (Koshimizu *et al*., 1994).

2.2.11 Hepatoprotective effect: Experimental rats treated with bitter leaf extract had considerably decreased plasma transaminase activity, according to a study on the impact of the extract against acetaminophen-induced hepatotoxicity (Johnson *et al*., 2015).

2.2.12 Cathartic activity: *Vernonia Amygdalina* methanol extract was revealed to have enhanced motility rate of charcoal meal in mice. It also stimulated gastric emptying (Awe *et al*., 1999).

2.3 Spermatogenesis

The process by which the male gamete, or spermatozoa, develops is referred to as spermatogenesis (de Krestser *et al*., 1998). Three steps make up spermatogenesis: Spermatogenial stem cells divide mitotically in the first stage, resulting in the production of Type A as well as Type B cells. While the Type B cells differentiate into primary spermatocyte, Type A cells replenishes the stem cells. In the second stage, the primary spermatocyte undergoes meiosis (meiosis 1) which divides into secondary spermatocyte, which then divide into haploid spermatids (Meiosis II). In the third stage, spermatids undergo spermiogenesis, a process that turns them into mature cells called spermatozoa (Sharma *et al*., 2018). From the tubule's base to its lumen, somatic sertoli cells inside the seminiferous tubules provide a niche in developing germ cells, supporting the process both qualitatively as well as quantitatively (Griswold, 1998). Sertoli cells interacts with germ cells by supplying them with nutrients coupled with paracrine compounds. Puberty marks the beginning of spermatogenesis, and lasts the rest of one's life (With minor variation in some species). However, their quality diminishes with age.

The regulation of spermatogenesis involves the interaction of several autocrine, paracrine, endocrine hormones coupled with the incorporation of a number of cellular processes (Ehmcke *et al*., 2006). Two pituitary gonadotropins, luteinizing hormone (LH) as well as follicle stimulating hormone (FSH) regulate the production of sperm.

2.3.1 Follicle stimulating hormone (FSH)

Follicle stimulating hormone is a glycoprotein polypeptide hormone produced by the adenohypophysis. Gonadotropin- releasing hormone (GnRH) triggers the release of FSH from the hypothalamus (Stamatiades and Kaiser, 2018). It is a heterodimer glycoprotein hormone made up of alpha as well as beta subunits. Both subunits are essential for physiological functions.

FSH is crucial for both male as well as female sexual development and reproduction. FSH induces the first meiosis division in addition to the production of secondary spermatocyte. It also initiates the production of androgen-binding protein, needed for spermatogenesis (Jiang *et al*., 2012).

2.3.2 Luteinizing Hormone (LH)

Luteinizing hormone production just like FSH is triggered by hypothalamic gonadotropin releasing hormone (GnRH). It has an alpha unit similar to that of follicle stimulating hormone (92 amino acids) coupled with beta subunits that is made of 120 amino acids. Luteinizing hormone binds to specific transmembrane receptor which are primarily located in the ovary as well as the testes and perform function which differs in male and females. In females, the production of androgens by follicular theca cells in the ovary is highly dependent on LH activity. In addition, it facilitates the required chain of events that leads to ovulation as well as aid the corpus luteum primary release of progesterone, which primes the uterus for potential implantation. In males, LH stimulates the production of testosterone. The testosterone plays in important role in libido regulation, development of male secondary sexual features (such as deeper voice, chest hair and muscle mass), spermatogenesis as well as fertility (Mooradian *et al*., 1987).

2.3.3 Male fertility

Infertility and issues with decreased fecundity have long been of concern and they continue to be a serious clinical issue that affect 8-12% of couples globally today. About 40 to 50% of infertility cases has been estimated to be a “male factor” infertility which results from abnormalities in fertility parameters. Male infertility refers to the inability of a mature adult to impregnate a fertile female (Sadock *et al*., 2003). Male factor infertility is characterized by sperm parameters inside adult men that are below the WHO standard values. These parameters include sperm concentration, motility as well as morphology. Other less linked variable of fertility includes semen volume in addition to seminal vesicle function.

Semen analysis also known as sperm count is a descriptive test used to assess the vitality coupled with the quality of male sperm. It is essential that this test is carried out in tandem with sperm functional assay, which assesses a spermatozoon's capacity to complete the task necessary to get to the ova, fertilize them coupled with potentially leading in live births (Sadock *et al*., 2003).

2.3.4 Normal male reproductive parameters

Sperm count -39 million/ejaculate

Sperm concentration -15million/ml

Sperm morphology -32%

Sperm vitality - 4%

Sperm volume – 1.5ml

PH – 7.2 (Mooradian *et al*., 1987).

2.3.5 Factors affecting male fertility

There has been mounting evidence of a prevalent decrease in the quality of male sperm during the past few years (Carlsen *et al*., 1992; Aitken, 2013). An investigation carried out by Jorgensen *et al*., 2012 on the sperm counts of men from different geographical group revealed a 50-60% decline in their sperm count (Jorgensen *et al*., 20001). This decline can be attributed to a number of factors ranging from environment, occupational factor along with lifestyle practices (Jurewicz *et al*., 2014; Sharma *et al*., 2013)

2.3.6 Lifestyle Practices

Life style practices that can alter sperm quality includes;

- Smoking
- Alcohol consumption
- Use of illicit drugs like Marijuana, cocaine, androgenic steroids (AAS), opiates (narcotics),
- Psychological stress
- Obesity
- Advanced paternal age
- Dietary practices
- Coffee consumption
- Other factors include testicular heat stress, sleep deprivation in addition to electromagnetic radiation exposure from cell phone use (Jurewicz *et al*., 2014; Sharma *et al*., 2013).

2.3.7 Smoking: Smoking-related toxins adversely affect sperm development as well as maturation. Smoking is known to be linked to leucocytospermia, a significant endogenous generator of reactive oxygen species (ROS). Smokers have higher amounts of ROS in their seminal that subjects the spermatozoa to oxidative stress and ultimately compromise male fertility. However, it is still unclear how these effects are brought about (Harlev *et al*., 2015). Meta-analysis encompassing males from different regions revealed that smoking results in a decrease in the quality of sperm in both fertile as well as infertile men (Li *et al*., 2011). Male smokers were found to have sperm concentration that were greatly lower than those of men who did not smoke (Vine *et al*., 1994). According to reports, men who smoked more frequently experienced a more drastic drop in sperm quality (Sharma *et al*., 2010). Preconception paternal smoking increases the likelihood of various morbilities within the progeny, which may be mediated by epigenetic alteration passed on through spermatozoa (Jenkins *et al*., 2017).

2.3.8 Alcohol: Alcohol effects on the male fertility cause an overall effect on the hypothalamus-pituitary gonad. It interferes with the synthesis of the various glycoprotein polypeptide coupled with the disruption of the activity of the leydig as well as the sertoli cells, thereby inhibiting spermatozoa maturation process. Direct alcohol consumption has been shown to adversely affect fertility parameters in a dose-dependent manner (Donnelly *et al*., 1999). With increasing amount of alcohol consumption, spermatogenesis appears to gradually decline (Pajarinen *et al*., 1996). Comparatively to non-drinkers, heavy drinkers were more likely to experience spermatogenic arrest (Pajarinen and Karhumen, 1994). Fertility parameters in men were reported to be negatively impacted by chronic alcohol consumption (Muthusami and Chinnaswany, 2005). On the other hand, moderate alcohol use was linked to increased testosterone levels (Jensen *et al*., 2014).

2.3.9 Recreational drugs: Illicit drugs that have detrimental effect on male fertility include marijuana, narcotics as well as methamphetamine. These drugs are known to have negative impacts on the HPG axis, testicular architecture in addition to sperm function. Low sperm parameters were reported in young male individuals who smoked cannabis frequently. These effects are heightened when mixed with other hard drugs. Spermatogenesis has been shown to be severely hampered by the economic liberalization of the endogenous cannabinoid system (ECS). Long term cocaine users were seen to have a high proportion of sperm with abnormal morphology, reduced concentration as well as motility (Bracken *et al*., 1990).

2.3.10 Obesity: Men who are obese or overweight have significantly lower sperm quality and are more likely to experience infertility. Due to the larger percentage of sperm with fragmented DNA, poorly shaped sperm coupled with sperm with low mitochondrial membrane potential (MMP), obese men are more likely to be infertile (Campbell *et al*., 2015). The degree of obesity effect on hormonal profile, sperm parameters, DNA damage as well as pregnancy outcomes may vary depending on the presence of other co-mordalities (Kahn and Brannigan, 2017).

2.3.11 Psychological stress: Li et al., 2011 reported that psychological stress was found to cause a decrease in male fertility parameters as well as sperm quality (Li *et al*., 2011).

2.3.12 Advanced paternal age: Analysis of semen parameters of healthy men across a broad age range (22-80 years) revealed the semen volume as well as the sperm motility decreased gradually continuously with age without a specific age threshold. As men age, the testis undergoes age-related morphological changes such as decline in the amount of germ cells, sertoli cells, as well as structural alterations which include narrowing of the seminiferous tubules. These changes invariably cause a decline in reproductive parameters (Gunes *et al*., 2016).

2.3.13 Diet: The quality of the semen is greatly influenced by diet coupled to nutrition. Eating a healthy diet such as one rich in fish, vegetables, fruits were observed to enhance sperm quality.

However, alcohol, full-fat diary items, processed meat were linked to lower sperm parameters (Salas-Huetos *et al*., 2017).

The exponential growth of industry in recent decade has led to the improvement of our daily lives, but it has also increased occupational as well as environmental exposure to various endocrine disrupting chemicals (EDCs) which plays a significant role in fertility disorders. (Santi *et al*., 2016)

.2.3.14 Occupational exposure

The risk of asthenozoospermia was considerably increased by occupational exposure to pesticides, while exposure to cement was connected to an increase in the risk of oligozoospermia. However, the study did not reveal a connection between solvent exposures, mechanical vibrations in addition to semen impairment (Daoud *et al*., 2017). However, another study found a link between exposure to lead, work related stress in addition to fertility (El-Helaly *et al*., 2010). Exposure to formaldehyde which is used in resin production, laboratories in addition to wood processing has shown to substantially decrease total as well as progressive sperm motility (Wang *et al*., 2015). Farm workers who were regularly exposed to organophosphate pesticide were observed to have lower sperm motility coupled with sperm immaturity (Miranda-Contreras *et al*., 2013). According to a study, occupational exposure to ethylene glycol-based chemicals correlated with poor sperm count, however the risk was substantially reduced after controlling the cofounders. Full-time firefighters were found to have an increased risk of infertility due to their frequent exposure to complex chemicals such as flame retardants as well as polyaromatic hydrocarbons (PAHs) (Peterson *et al*., 2018).

2.3.15 Environmental exposure

Environmental pollution has emerged as a significant contributor in the global decrease in male reproductive parameters. Semen quality is affected by a number of modifiable environmental factors including air pollution, pesticides coupled with hazardous chemicals (Kumar and Singh, 2022).

2.3.16 Air pollution

The primary cause of air pollution are emissions from motor vehicles, homes as well as farms. The primary air pollutants that have a negative impact on human health are particulate matter, nitrogen oxides, carbon monoxide, in addition x-rays. Tiny liquids or solid droplets are parts of the airborne particulates matter can be inhaled which can cause detrimental consequences on one's health. Sulfur dioxide (SO2) exposure had a detrimental effect on sperm quality, according to a study that evaluated the association between various gaseous pollutants coupled with semen quality. Additionally, it was observed that both sulfur dioxide and nitric dioxide had a detrimental effect on sperm parameters, which were believed to be more combative in the early phase of spermatozoa production (Wang *et al*., 2020). According to the study, tollgate employees who were exposed to a lot of vehicle pollution had more damaged sperm chromatin and fragmented DNA than their colleagues who were not exposed. Hence, leading the researchers to the conclusion that exposure to car exhaust can have significant genetoxic effect on human spermatozoa (Calogero *et al*., 2011).

2.3.17 Exposure to harmful chemicals

All around the globe, people are constantly exposed to chemicals which are detrimental to the body physiology. According to recent research, male reproductive organs are one of the primary sites for injuries brought on by the exposure to environmental toxins that causes male infertility (Dissanayake et al., 2019). Examples of these chemicals include dioxins, pesticides as well as herbicides.

2.3.18 Heavy metals

Non-essential heavy metals such as lead, arsenic in addition to barium are examples of pervasive environmental toxins that affect male fertility. These metals were found to be strongly connected with an elevated risk for lower sperm viability coupled with abnormal sperm morphology in seminal plasma and blood respectively (Sukhn *et al*., 2018). Heavy metals affect male fertility by increasing the synthesis of free radicals, which leads to lipids peroxidation coupled with sperm DNA damage (Jamalan *et al*., 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Assembling of Plant Sample

The leaves of paw-paw and bitter leaves plant (*Carica Papaya and Vernonia Amygdalina*) were collected between January and February 2022 from the botanical garden of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. The plants were validated by Dr H. Akinibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. The leaves samples were initially allowed to dry naturally for two weeks at room temperature for two weeks before being thoroughly dried inside an oven for 1 hour at 40^oC. The dried samples were then ground into a fine powder with an electric mill before being stored in airtight containers (Oshomoh and Obaro, 2019a).

3.1.2 Preparation of Extract

Using cold maceration method, one kilogram (1 kg) of the respective powdered plants were extracted with N-hexane solvent. The powdered samples were placed in the ratio of 1:1 a gas jar and filled up to three-quarter (3/4) with absolute N-hexane. This mixture was kept inside a dark cupboard and vigorously shaked as often as possible. Three days after, the solution was filtered using a cheese cloth. The resultants were then concentrated to dryness in a vacuum at 40^{o}C with a rotary evaporator. The concentrates were dried completely in an oven at 40^{o} C. The yield percentage was obtained concerning used dried powder for extraction. The specific weight of extracts was diluted in distilled water to obtain a stock solution from which dilutions were made and calculated doses were administered to the animals during the various experimental procedures (Oshomoh and Obaro, 2019b).

3.1.3 Laboratory Animals

Matured male albino Wistar rats, were purchased from the Animal House, Department of Pharmacology/ toxicology, Faculty of Pharmacy, University of Benin, Benin City and were housed (5 mice/rats/cage) in polypropylene cages lined with wood shaven, renewed every day and had free access to tap water and pelletized top feed. The animals were handled in accordance with standard procedure and exposed to a 12 hours light-dark cycle.

3.1.4 Acute Toxicity Study

The Acute toxicity study was conducted in accordance with OECD (Organization of economic co-operation development), 2008a guidelines. Six (6) mice (comprising of 3 male and 3 female) recieved 5000 mg/kg per body weight of the bi-herbal extract and observed for 72 hours for possible signs of toxicity, mortality or morbidity.

3.1.5 Male Reproductive Toxicity Study

Reproductive toxicity study was carried out by up method. 2000 mg/kg of the extracts were administered to the animals and monitored for 28 days for any indications of toxicity, mortality and morbidity. Twenty-four (24) hours after the administration of the last doses, all the animals were sacrificed, male reproductive organs, blood and sperm samples were collected, hormonal assay, sperm analysis as well as histopathology studies were evaluated (Olorunnisola *et al.,* 2012).

3.1.6 Fertility Study Fertility effects of the bi-herbal were carried out on male albino rats using methods of Orieke *et al*., (2019) respectively.

3.2 Parameters assessed in Male Albino Rats

3.2.2 Body Mass (g)

For the evaluation of body weight, the rats' weight was recorded on day zero (0) of administration of extracts as well as on weekly basis.

3.2.3 Assessment of Male Reproductive Organs

After sacrifice, the rats were subjected to laparotomy after which the testes, prostate, seminal vesicles coupled with the right epididymis were isolated. These reproductive organs were then freed from contiguous fatty tissues followed by weighing in an analytical weighing balance then relative weight were respectively calculated (organ mass per 100 g of body mass). Organs were further inspected macroscopically for valuation of variations in colour, appearance, shape in addition to the sizes.

3.2.4. Rate of Fertility

Sperm was isolated from the left epididymis. A section of the sperm collected was placed in 0.5 mL of saline solution; 20 mL of distilled water was then used to dilute it. A portion of the homogenate was placed in a hemocytometer to determine the total sperm count. The number of spermatozoa was attained by an average of two counts, consistent with the lower as well as the upper fields.

CHAPTER FOUR

RESULTS

4.1. Acute Toxicity Study

After 72 hours of administration of the bi-herbal extract (5000 mg/kg) revealed no mortality, loss of cognitive/ loss of agility or any physical morphology associated with toxicity was observed in all the groups (Table 4.1).

Table 4.1: Acute effect of bi-herbal formulation on albino rats after 72 hours administration of single-dose (5000 mg/kg) of extract.

Group(s)	Dose (mg/kg)	Cognition	Agility	Signs of Toxicity such as Grooming, nausea, writhing,	Mortality after 72 hours of administration
Distilled H_2O	2 ml/kg	Normal	Normal	None	0/6
Bi-herbal Extract	5000	Normal	Normal	None	0/6

4.2. Effect of the bi-herbal formulation of *bitter leaf and paw-paw leaves* on weight index of males Wistar rats after 28 days treatment

After 28 days of treatment, noteworthy reduction in weight was recorded in all the extract treated groups. The N-hexane extract had the lowest weight (279.2 ± 9.48) when compared to control 358.8 ± 2.62. On sacrificing however there was no substantial difference in reproductive organ weight between the control and treated groups (Table 4.2 and 4.3).

Table 4.2: Effect of **t**he bi-herbal formulation of *bitter leaf and paw-paw leaves* on weight index of males Wistar rats treated for 28 days

		Body Weight (g)			
Treatments (mg/kg)	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	180.8 ± 3.28	185.2 ± 4.17	186.9 ± 3.54	187.6 ± 4.71	190.6 ± 3.52
Proviron 25	182.0 ± 3.57	180.4 ± 4.05	182.6 ± 3.45	185.7 ± 4.10	187.0 ± 5.96
Bi-herbal 100	184.5 ± 3.45	180.2±2.76 [a]	177.8 ± 1.16 [b]	167.8 ± 1.16[d]	160.8 ± 1.16[d]
Bi-herbal 500	183.7 ± 2.98	175.8±3.55 [a]	169.8 ± 2.74 [b]	159.8 ± 4.71[d]	154.8 ± 3.62 [d]

Values are expressed in mean ± SEM as compared with control, n=5. [a] = ($p≤0.05$), [b] = ($p≤0.01$), [d] = ($p≤0.0001$)

Table 4.3: Effect of **t**he bi-herbal formulation of *bitter leaf and paw-paw leaves* on reproductive organ weight and relative organ weight index of males Wistar rats treated for 28 days

Organs Weight (g)	**Control**	**Proviron 25 mg/kg**	**Bi-herbal 100 mg/kg**	**Bi-herbal 500 mg/kg**
Right testicle	1.8 ± 0.7	1.9 ± 0.5	1.3±0.6	1.2±0.9
Left testicle	1.9 ± 0.8	2.1 ± 0.6	1.1 ± 0.9	1.0±0.6

Seminal vesicle	1.6 ± 0.6	1.7 ± 0.7	1.3± 0.4	1.1±0.8
Right epididymis	0.9 ± 0.2	1.1 ± 0.3	0.7±0.2	0.5±0.3
Prostate	0.7 ± 0.3	1.0 ± 0.4	0.4±0.1	0.3±0.1

Relative weight (%)	**Control**	**Proviron 25 mg/kg**	**Bi-herbal 100 mg/kg**	**Bi-herbal 500 mg/kg**
Right testicle	0.8 ± 0.4	0.9 ± 0.3	0.5±0.2	0.4±0.2
Left testicle	0.9 ± 0.3	1.0 ±0.2	0.6±0.3	0.5±0.1
Seminal vesicle	0.6 ± 0.3	0.8±0.4	0.5±0.4	0.4±0.2
Right epididymis	0.6 ± 0.2	0.7± 0.3	0.4±0.2	0.3± 0.1
Prostate	0.18± 0.03	0.20±0.04	0.09±0.04	0.06 ± 0.03

4.4. Effect of the bi-herbal formulation on sperm cell quality of male albino Wistar rats

Total sperm count was considerably lowered in all the treatment groups. Decrease in the progressive motility and normal cell counts were respectively recorded (Table 4.4).

Table 4.4: Effect of the bi-herbal formulation on sperm quality of adult male albino Wistar rats

Study	**Total**	**Progressive**	**Non**	**Immotile**	**Normal**	**Abnormal**	**Sperm**

Group (mg/kg)	sperm Cell count X10^6 cells/mm^3	Motility (%)	Progressive Motility (%)	(%)	Morphology (%)	Morphology (%)	P^H/Colour
Control	380.6±4.8	90	05	05	90	10	6/Cream
Prov.25	390.9±5.9	90	05	05	70	30	6/Cream
BHF100	270.3±6.7^d	70	15	15	50	50	7/white
BHF500	250.6±5.4^d	50	30	20	40	60	8/white

Results are presented in mean± SEM, n=5, ^{d} =*p≤0.0001*

Normal sperm cell range in rats=350-500 (X10^6 cells/mm^3)

Oligozoospermia ≤ 100(X10^6 cells/mm^3)

Key: BHF= Bi-herbal Formulation

Prov = Proviron

CHAPTER FIVE

5.1 DISCUSSION OF RESULT

5.1.1 Acute effect of bi-herbal formulation

This study was carried out to evaluate the effect of N-hexanoic bi-herbal formulation extract of paw-paw and bitter leaf on the reproductive system of Wistar albino rats. Table 4.1 shows the acute effect of the bi-herbal formulation. The primary objective of acute toxicity studies is to pinpoint the dose that, when administered once or in multiple doses, will produce mortality or major toxicology consequences. Additionally, they serve to influence future trials' dose requirement (Chinedu *et al*., 2013). Previous studies done to evaluate the acute toxicity of *Carica papaya* and *Vernonia amygdalina* individually, did not yield any toxic effect, hence these extracts are considered to be safe when administered individually. This acute toxicity test was carried out to determine if the bi-herbal formulation which comprises of both *Carica papaya* and *Vernonia amygdalina* will cause any toxic effect in the test animals. 5000mg/kg of the extract was administered to the rats, and they were observed for signs of grooming, nausea, writhing, decline in agility and cognitive function or toxicity- related mortality. After 72 hours of administration of the bi-herbal extract (5000 mg/kg), no mortality, loss of cognitive/ loss of agility or any physical morphology associated with toxicity was observed in all the treatment groups. Hence LD50 is greater than 500mg/kg.

5.2 Effect of the bi-herbal formulation on weight index of males Wistar rats

Results presented in Table 4:2 shows the weight of the experimental animals at different intervals (weekly) during the course of the trial. During the experiment, two doses (100mg/kg and 500mg/kg) were administered). Proviron 25, a known male fertility drug was used as the standard drug. Proviron also known as mesterolone is an orally active compound that belongs to a class of drugs known as androgen, or 'male sex hormone'. It is commonly used in the treatment of hypogonadism in men, a condition that stems from low testosterone level. It functions by enhancing and supplementing the quantity of male hormones the body naturally generates (Michael steward, 2019). Two doses (100mg/kg and 500mg/kg) of the bi-herbal formulation were administered to the rats. A significant reduction in the body weight of the animals, from an original weight of 184g and 183g to 160g and 154g respectively after 4 weeks of treatment was observed. The primary enzyme involved in the digestion of fat, pancreatic lipase, hydrolyzes triglycerides, which makes up the majority of dietary lipids into free fatty acids and monoglycerides (Mukherjee, 2003). Free fatty acids are released into the blood system, where they are distributed to the liver and adipose tissue, causing lipid buildup and ultimately resulting in obesity. Inhibition of pancreatic lipase reduces digestion and the absorption of fat; therefore, it is one of the most common treatment for obesity. According to the findings of a study conducted by Lunagariya and colleagues, papaya suppressed pancreatic lipase activity. This action is advantageous since it delays the absorption of fats, which result in a reduction in body weight (Lunagariya *et al*., 2014). A study carried out using *Vernonia Aygdalina* also revealed similar outcome. The body weight of Vernonia amygdalina treated rats was found to have substantially reduced days after receiving the extract. (Atangwho and Mariam, 2012). Hence, the great reduction in the body weight of the animals treated with the bi-herbal formulation can be attributed to the combined antiobesity activity of both plants. Therefore, this extract can be used for weight loss.

5.3: Effect of the bi-herbal formulation of on reproductive organ weight and relative organ weight index of males Wistar rat

There was no noteworthy difference in the organ weight and relative organ weight of the extract when compared to that of the control group.

5.4 Effect of the bi-herbal formulation on sperm cell quality of male Wistar rats

The result in Table 4.5 revealed a decrease in the sperm count in all the treatment groups. Decrease in the progressive motility and normal cell counts were also observed. The result is in support of the study carried out by Chinaka and Rita, 2019 which revealed a similar reduction in male fertility comparable to the normal control. However, in contrast to our test result, a study carried out by Risikat *et al*., 2020, revealed that a low dose of *Vernonia amygdalina* (Bitter leaf) improved the sperm count of immunosuppressed rats significantly. At both high and low dose of *Vernonia amygdalina* caused an increase in sperm parameters was observed. Although the exact by mechanism by which this extract reduce sperm count is unknown, prior research has demonstrated that antimalarial remedies typically have antifertility effect. Malaria has been treated using pawpaw extract in the past, (Titanji *et a*l., 2008) as a result, there may be a connection between its antimalarial and anti-fertility properties.

5.4 CONCLUSION

The result of the study shows that the bi-herbal formulation of *Carica papaya* and *Vernonia amygdalina* had a negative effect on the sperm and reproductive parameters which is crucial for fertility, thus the extract poses as a likely candidate for contraception. It is also effective in weight loss.

5.5 RECOMMENDATION

I recommend that more research should be carried out on bi-herbal formulation of *Carica papaya* and *Vernonia amygdalina*, to explore other medicative potentials of this plants. Campaigns should be organized to enlighten people against the use of plants formulation whose therapeutic effects has not been investigated, as interaction of the plant phytochemical may yield a toxic effect.

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