ANTIFERTILITY EFFECT OF BIHERBAL FORMULATION (NETUGA) ON SOME REPRODUCTIVE PARAMETERS

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BENIN CITY

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A PROJECT WRITTEN IN THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY AND SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELORS (B.Sc.) DEGREE IN THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

MAY, 2024

CERTIFICATION

We certify that this research titled ANTIFERTILITY EFFECT OF BIHERBAL FORMULATION (NETUGA) ON SOME REPRODUCTIVE PARAMETER was carried out by **Oluwafumike AKINMUTOLA (MISS)** with Matriculation number **LSC1807171** in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate this success story to God Almighty, for his grace upon my life.

ACKNOWLEDGEMENTS

I am very grateful to God Almighty for His protection and favour throughout this programme.

My profound gratitude goes to my wonderful supervisor, Dr. P. O. Obaro and co-supervisor Dr. (Mrs.). O. E. Obaro-Onezeyi for their love, support and parental criticisms.

I am grateful to the entire staff of Science Laboratory Technology Department especially the present and immediate past HOD - Prof. E.O. Oshomoh and Prof. J.O. Osarumwense. To the project coordinator – Mrs. P.O. Omozuwa, I am indeed grateful.

My deep gratitude goes to my mum, dad and my siblings Mrs Akinmutola Elizabeth, Mr Akinmutola meshell and my siblings: Mr Abayomi Akinmutola, Mrs imuntenyan Oluwabunmi , Mrs Osamuyi Opeoluwa, and Miss Oluwabukola Akinmutola.

To all my friends, course mates and a host of others.

My sincere appreciation to all whom in one way or the other contributed to this success story especially to myself I say a big thank you.

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CHAPTER ONE

1.0 BACKGROUND STUDY

1.1 Fertility

Fertility is the natural capacity to conceive a child. Some people find fertility difficult to achieve and around 17.5% of the adult population – roughly 1 in 6 people worldwide experience infertility, showing the urgent need to increase access to affordable, high-quality fertility care for those in need (World Health Organization, 2023).

1.2 Infertility

According to the National Institute of Health (2017), Infertility is defined clinically as not being able to achieve pregnancy after 1 year of having regular, unprotected intercourse, or after 6 months if the woman in 35 years of age.

1.3 Subfertility

This is sometimes used to mean the same thing as infertility, but they are slightly different. Subfertility means that pregnancy is likely to occur without medical intervention, but it takes longer than usual (Gnoth *et al*., 2005).

Fertility problems does not discriminate and is a subject of discussion among both genders. It can cause significant distress, stigma, and financial hardship, affecting people's mental and psychosocial well-being. However, not being able to conceive is not always linked to a disease.

1.4 Fertility in Females

In many countries of the world, male fertility is higher than female (United Nations, 2017). It is at least 15 percent higher and male fertility is also later than female fertility. The mean age at

fatherhood is on average 35 years, compared to 29 years for the mean female age at childbearing. A third of the time, fertility problems are found in men, a third of the time in women and a third of the time in both partners. A thorough fertility evaluation will take into account the physical health and medical history of both partners.

The fertility rate in Nigeria is on a slow decline. This is despite ranking 16th on a list of countries with the highest fertility rates. Fertility rate is defined as the average number of children that would be born to a woman over her lifetime if she were to live from birth until the end of her reproductive life, and if she were to experience adequate age-specific fertility rates through her lifetime. The average global fertility rate is 2.4 children per woman, while Nigeria's average fertility rate is 4.67 as at April 2021. However, this is in comparison to 5.3 in 2019, 5.1 in 2017, 6.2 in 1990, and 6.8 in 1980. Recent forecasts predict that Nigeria's fertility rate will be 1.7 in the year 2100. There are multiple concomitant factors that contribute to this decline; these include but are not limited to the following: The decrease in male fertility as a result of poorly treated sexually transmitted infections (iSTIs), epidemic diseases like malaria, Schistosomiasis and viral infections, hormonal abnormalities, consumption of excessive alcohol, tobacco smoking, exposure to pesticides and heavy metals, and reports of higher concentrations of aflatoxin B1 (AFB1) in the semen of infertile Nigerian men. Another predisposing factor is the increase in female education; increased use of contraception and family planning; and the decline of the economy

1.5 Disorders of Female Infertility.

There are many causes of infertility, and sometimes, there isn't a simple answer as to why couples are not getting pregnant.

1.6 Infertility causes for women

Ovulation disorders which is the most common cause of infertility in people with ovaries. Ovulation is the process in which your ovary releases an egg to meet sperm for fertilization. Other factors that can contribute to female infertility includes: Endometriosis,

Structural abnormalities of the vagina, uterus or fallopian tubes, Autoimmune conditions like celiac disease or lupus, Kidney disease, Pelvic inflammatory disease (PID), Hypothalamic and pituitary gland disorders, Polycystic ovary syndrome (PCOS), Primary ovarian insufficiency or poor egg quality, Sickle cell anemia, Uterine fibroids or uterine polyps, Thyroid disease, Prior surgical sterilization (tubal ligation or salpingectomy), Genetic or chromosomal disorders, Sexual dysfunction, Surgical or congenital absence of the ovaries, Infrequent or absent menstrual periods.

1.8 Drugs to treat Female Infertility

Infertility can be cured, but it depends on the cause. In 85% to 90% of cases, lifestyle modification, medication, ART or surgery can treat infertility and allow a person to conceive. Treatment for infertility often depends on the cause and goals of therapy. Often, lifestyle changes or improving the frequency and timing of intercourse can improve the chances of pregnancy. Treatment can also include a combination of methods.

1.9 Treatments for infertility in women

These include lifestyle modification involving either gaining or losing weight, stopping smoking or using drugs, and improving other health conditions can improve the chances of pregnancy. Medications such as Fertility drugs stimulate the ovaries to ovulate more eggs, which increases

chance of getting pregnant while surgery can open blocked fallopian tubes and remove polyps, fibroids or scar tissue.

There are lots of drugs, but here are the basics on the ones that are most commonly prescribed.

- **Clomiphene citrate**.

This drug stimulates ovulation by causing the pituitary gland to release more follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which stimulate the growth of an ovarian follicle containing an egg. This is generally the first line treatment for women younger than 39 who don't have polycystic ovary syndrome (PCOS).

- **Gonadotropins**.

These are injected treatments that stimulate the ovary to produce multiple eggs. Gonadotropin medications include human menopausal gonadotropin or hMG (Menopur) and FSH (Gonal-F, Follistim AQ, Bravelle).

Another gonadotropin, is the human chorionic gonadotropin (Ovidrel, Pregnyl), is used to mature the eggs and trigger their release at the time of ovulation. Concerns exist that there's a higher risk of conceiving multiples and having a premature delivery with gonadotropin use.

- **Metformin**.

This drug is used when insulin resistance is a known or suspected cause of infertility, usually in women with a diagnosis of PCOS. Metformin helps improve insulin resistance, which can improve the likelihood of ovulation.

- **Letrozole**

This belongs to a class of drugs known as aromatase inhibitors and works in a similar fashion to clomiphene. Letrozole is usually used for woman younger than 39 who have PCOS.

- **Bromocriptine and Cabergoline**

This is a dopamine agonist, and might be used when ovulation problems are caused by excess production of prolactin (hyperprolactinemia) by the pituitary gland.

1.10 Female Reproductive Hormones

Hormones are a diverse class of molecules that influence complex activities throughout the body. They are commonly categorized as peptides and proteins (Hiller-Sturmhöfel and Bartke, 1998) and are produced in the gonads and adrenal glands, as well as various other tissue and cell types, including those within the placenta and brain (Schiffer *et al*., 2019). The main reproductive hormones estrogen, testosterone, and progesterone are pivotal in sexuality and fertility. They are essentially responsible for pregnancy, puberty, menstruation, menopause, sex drive, sperm production and more. These hormones are produced in the ovaries (in females) and testes (in males). The pituitary gland produces, stores and stimulates other reproductive hormones such as: the Human Chorionic Gonadotropin (HcG), Prolactin, Luteinizing Hormone (LH) and the Follicle-Stimulating Hormone (FSH).

1.11 AIM OF STUDY

The aim of this study was to evaluate and investigate the effect of bi-herbal formulation of Garlic and turmeric on female reproductive parameters and organs

1.12 OBJECTIVE OF STUDY

The objectives of this study were to study;

- The Formulation of the bi- Herbal mixture of Garlic and turmeric on female reproductive parameters and organs
- The effect of the bi- Herbal mixture of Garlic and turmeric on female reproductive parameters and organs using standard methods.

CHAPTER TWO

LITERATURE REVIEW

2.0. *Allium* sativum (Garlic)

Allium sativum (Garlic), a member of the Amaryllidaceae family is an ancient civilized plant that originated from the Asian continent between the Mediterranean and China over 600 years. Garlic close relatives include the onion, shallot, leek, chive, Welsh onion, and Chinese onion. It is native to South Asia, Central Asia and northeastern Iran and has long been used as a seasoning worldwide, with a history of several thousand years of human consumption and use. It was known to ancient Egyptians and has been used as both a food flavoring and a traditional medicine. China produced 73% of the world's supply of garlic in 2021 (FAOSTAT, 2023). The plant is a bulb growing to 25-70 cm with hermaphrodite flowers. The bulbs of garlic range in color from white to pink having a pungent scent and fragrant flavor. Each segment of a garlic bulb is called a clove. There are about 10–20 cloves in a single bulb, give or take. The Leaves, cloves, oil and aged extract of garlic have been used in traditional medicine of Iran and other countries for a long time. The therapeutic effects of garlic are mainly due to the impressive activity of its bioactive compounds. Spiritually, garlic was prized as a protective agent. This is perhaps the origin of our common association with garlic as a deterrent to vampires. It was one of several plants in ancient Greece that was worn to guard against illness and negative energies, including theft and possession by evil spirits. Both sailors and soldiers were known to wear protective cloves, both for spiritual protection and in case of sickness.

Ancient Egyptians used it as a form of currency, and hieroglyphics of its medicinal benefits have been found transcribed on ancient temple walls and on papyrus dating back to 1500 BC. Arabian

herbalists used garlic to respond to abdominal pain, infantile colic, diarrhea, diabetes, eye infections, snakebites, and even tuberculosis. African herbalists used garlic to treat respiratory and helminthic infections, as well as garlic oil in drops to respond to childhood ear infections. In North America, Native Americans used garlic for a variety of healing purposes and consumed it on a regular basis. In China and Europe garlic was used against plagues and infectious diseases as well as lung complications. In 1800's America it was common for physicians to recommended garlic inhalation as a treatment for TB. During World War I, garlic poultices were used to prevent wound infections, and by WWII the plant had a reputation as "Russian penicillin." Physicians in many Western Countries including American actually relied on garlic specifically as an antihypertensive agent up until the late 1950's when potent pharmaceuticals became more widely available. The first scientific studies on garlic were reported by Louis Pasteur, who attributed the plant as having anti-bacterial properties (Block, 1985).

Garlic is easy to grow and can be grown year-round in mild climates. While sexual propagation of garlic is possible, nearly all of the garlic in cultivation is propagated asexually by planting individual cloves in the ground. In colder climates, cloves are best planted about six weeks before the soil freezes. The goal is to have the bulbs produce only roots and no shoots above the ground. Harvest is usually in the late spring or early summer. Garlic plants can be grown closely together, leaving enough space for the bulbs to mature, and are easily grown in containers of sufficient depth.

2.1. Active Constituents of Garlic

Garlic contains at least 33 sulfur compounds, several enzymes and 17 amino acids. Additional constituents of intact garlic include steroidal glycosides and lectins (Fenwick and Hanley, 1985). The phytochemicals responsible for the sharp flavor of garlic are produced when the plant's cells

are damaged. When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulfur-containing compounds stored in the cell fluids (cytosol). The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Some of the compounds are unstable and continue to react over time (Jones *et al*., 2004).

Sulphur Compounds in Garlic

It contains higher concentrations of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic's pungent odour and many of its medicinal effects. The abundant sulfur compounds in garlic are also responsible for turning garlic green or blue during pickling and cooking. The sulfur compounds in garlic accounts for approximately 2.3% and it has the water soluble components and the oil soluble components. The water soluble compounds include allin, cysteine, S-Allyl-Cysteine (SAC), and S-Allyl-Mercaptol-Cysteine (SAMC). The oil soluble sulphur compounds includes. Allicin, Diallyl sulfides (DAS), Diallyl disulfides (DADS), Diallyl trisulfides (DATS) and Ajoene (Kovarovic *et al*., 2019).

Non-sulphur Garlic Phytochemicals

Although little is known about their bioavailability and biological activities, non-sulfur garlic phytochemicals, including flavonoids, steroid saponins, organoselenium compounds, and allixin, likely work in synergy with organosulfur compounds. Garlic is abundant in phenolic compounds such as caffeic acid, p-coumaric acid, ferulic acid, and their respective derivatives. However, flavonoids are the most common plant antioxidant compounds, alongside quercetin, kaempferol, and apigenin, typically found in garlic. Significantly, the levels and varieties of antioxidant compounds in garlic may differ depending on factors such as garlic variety, cultivation conditions, and processing methods

Nutritional composition of garlic

Water (58.6g), Energy (623 KJ), Protein (6.36g), Total fat (0.5g), Ash (1.5g), Carbohydrates (33.1g), Total dietary fiber (2.1g), Sugar (1g). Calcium (181 mg), Iron (1.7mg), Magnesium (25mg), Phosphorus (153 mg), Potassium (401 mg), Sodium (17mg), Zinc (1.16mg), Copper (0.299 mg), Manganese (1.67 mg) and Selenium (14.2 µg) Vitamin C (31.2mg), Thiamin (0.2mg), Riboflavin (0.11mg), Niacin (0.7mg), Pantothenic acid (0.596 mg), Vitamin B6 (1.24 mg), Total Folate (3 µg), Total Choline (23.2mg), Vitamin A (9 IU), Vitamin E (0.08 mg), Vitamin K (1.7 µg).Total saturated fatty acids (0.089 g), Total monounsaturated fatty acids (0.011 mg), Total polyunsaturated fatty acids (0249 g). Tryptophan (0.066 g), Isoleucine (0.217 g), Leucine (0.308 g), Lysine (0.273 g), Methionine (0.076 g), Cystine (0.065 g), Phenylalanine (0.0183 g), Tyrosine (0.081g), Valine (0.291 g), Arginine (0.634 g), Histidine (0.113 g), Alanine (0.132 g), Aspartic acid (0.489 g), Glutamic acid (0.805 g), glycine (0.2 g), Proline (0.1g), Serine (0.19g). USDA (2022)

Plate 2.1: Garlic Bulb (Source: Hasib *et al*., 2016)

2.2. Taxonomy of Garlic

Scientific Classification of Garlic

Kingdom	Plantae
Subkingdom	Tracheobionte
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Equisetopsida
Subclass	Magnolioliidae
Superorder	Lilianae
Order	Asparagales
Family	Amaryllidaceae
Genius	Allium
Species	A. Sativum

(So *et al.,* 2021)

Garlic belongs to the Allium genus, which includes more than 750 species divided into more than 60 taxonomic groups. It is cultivated in many countries throughout the world for the bulb and used as a spice and functional food. The plant vegetatively propagates. With roughly 1.58 million hectares under cultivation worldwide, garlic is the second-most significant Allium crop, after onions. The name *Allium sativum* is derived from the Celtic word all, meaning burning or stinging, and the Latin sativum meaning planted or cultivated. The English name is derived from Anglo-Saxon gar-leac, meaning spear plant (in reference to the shape of its leaves). Over 300 garlic varieties have been recognized, collectively categorized into two major types: soft-neck garlic *(Allium sativum var. sativum)* and hard-neck garlic *(A. sativum var. ophioscorodon).* Taxonomically, the hard-neck type produces flowering pods, which are not apparent in the soft-neck garlic species. Even though garlic has demonstrated significant genetic variation in terms of

morphology, the genotype has almost no effect on nutritional and functional properties (Abedi *et al.,* 2013)

2.3. Pharmacological Benefits of Garlic and Mechanism

Anti-viral Properties

Allicin and its derivatives, ajoene, allitridin and garlicin, were found to be the most promising organosulfur compounds (OSCs) of garlic and are responsible for several of garlic's therapeutic activities including the prevention of viral infections. Studies conducted in in-vivo and in-vitro test models clearly demonstrate antiviral potential of garlic and its OSCs against a wide range of viruses; for example from the Adenoviridae, Arteriviridae, Coronaviridae, Flaviviridae, Flaviviridae, Herpesviridae, Orthomyxoviridae, Picornaviridae, Paramyxoviridae, Poxvirus, Rhabdoviridae and Retroviridae family. Garlic has been used for centuries as an ethnomedicinal plant to treat infectious diseases. It has been reported that fresh garlic ingestion or intravenous preparation of its extracts is used to treat various viral infections or cryptococcal meningitis patients, respectively, in China (Tsai *et al*., 1985).

Garlic has been reported to have antiviral activity against human, animal and plant viral infection. Research shows that crude GE and its OSCs exert their antiviral activity through interaction with the viral cell surface charge molecule and subsequently block or inhibit viral entry into host cells. Therefore, blocking viral entry and fusion into host cells, inhibiting viral RNA polymerase, reverse transcriptase, and viral replication, as well as enhancing host immune response were the major pathways for antiviral activity of garlic and its OSCs. Immunomodulatory activity was shown to elevate the innate immune response via macrophage and NK cells, as well as enhance adaptive immunity through T cells, B cells and anti-inflammatory cytokines (Shojai *et al.,* 2016).

Randomized clinical trials utilizing a variety of commercial garlic preparations revealed a prophylactic effect of garlic in the prevention and treatment of a number of viral infections in human including the common cold and flu, viral induced hepatitis and warts. Improved immune response was claimed to be responsible for these effects

Anti-bacterial Properties

Garlic is one of the popular spices added to food to enhance the flavor, and it has been used in different cultures and traditions around the world to treat bacterial infections for centuries. Several studies have evaluated the antibacterial activity of various garlic preparations such as crude or fresh garlic extract (FGE), and garlic paste. The antibacterial activity of garlic paste and FGE against commensal and pathogen enteric bacteria such as Escherichia coli, E. coli O157:H7, Salmonella species, *Shigella species, Vibrio species, Campylobacter species, Listeria monocytogenes, Enterobacter,* and *Enterococcus species, Lactobacillus acidophilus, Staphylococcus aureus, Streptococcus species,* and *Clostridium difficile* has been reported by various laboratories (Johnson and Vaughn, 1969; Kumar and Berwal, 1998; Ross *et al.,* 2001; Gupta and Ravishankar, 2005; Vuddhakul *et al.,* 2007; Lu *et al*., 2011b; Jain *et al*., 2015a; Roshan *et al*., 2017). These studies suggest that garlic consumption could help in preventing food poisoning. In addition, various studies have evaluated the impact of garlic and its organosulfur compounds on the gut microbiome. Garlic was found to positively influence the gut microbiome and protect the gut microbiome damage from high-fat diet (Chen *et al.,* 2018). Supplementing feed of farrowing sows and European bass with GO decreased pathogenic microbes from the gut microbiome (Rimoldi *et al.,* 2020; Satora *et al.*, 2020). In a small-scale clinical trial, aged garlic extract supplementation for 3 months increases the richness and diversity of the gut microbiome with increase in *Lactobacillus* and *Clostridium species* (Ried *et*

al., 2018). All the studies indicate that garlic and its compounds have a positive effect on gut microbiome composition and richness.

Another study also found that FGE was effective against MDR strains of *E. coli,* Pseudomonas aeruginosa, *Klebsiella pneumoniae, Serratia marcescens*, and MRSA in both in vitro and in vivo assays (Farrag *et al*., 2019). Raw garlic extract and commercial garlic tablets displayed synergistic effects against *H. pylori* when used along with omeprazole whereas no such effect was noticed when used in combination with amoxicillin, clarithromycin, and metronidazole (Jonkers *et al.,* 1999b). The principal phytochemicals that exhibit antibacterial activity are oil-soluble organosulfur compounds that include allicin, ajoenes, and allyl sulfides. The organosulfur compounds of garlic exhibit a range of antibacterial properties such as bactericidal, antibiofilm, antitoxin, and anti-quorum sensing activity against a wide range of bacteria including multi-drug resistant (MDR) strains. The reactive organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes and compromise the integrity of the bacterial membrane. In the food industry, fresh garlic oil is utilized as a natural antioxidant, flavoring ingredient, and antibacterial, especially in gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* in processed chicken and meat products.

The World Health Organization (WHO) has recognized the development of antibiotic resistance as a global health concern and emphasizes antibiotic stewardship along with the urgent need to develop novel antibiotics. Multiple antibacterial effects of organosulfur compounds provide an excellent framework to develop them into novel antibiotics (Jang *et al.,* 2018).

Anti-fungal Properties

Dermatophytes are a group of keratinophylic filamentous fungi infecting the skin and skin appendages of humans and animals. One of the most frequently isolated dermatophytes is *Trichophyton rubrum*. Antifungal agents such as imidazole and triazole drugs have been used in the treatment of various fungal infections. However, resistance to these drugs and the appearance of their side effects as well as their toxicity due to the administration of these drugs have been reported. As an alternative, plant extracts in herbal medicine have been used in the treatment of dermatophytosis. One of the plant extracts which has been used in this way is garlic. Previous in vitro studies indicated the effects of garlic extract on the inhibition of the growth in a large number of yeasts including *Candida spp*., some fungi such as *Coccidioides immitis*, and also dermato-phytic fungi *T. rubrum, T. mentagrophytes, T. verrucosum, Microsporum canis* and *Epider mophyton flocossum*. A sulphur-containing compound in garlic, known as di-allyl thiosulfinate (allicin), is the active component in inhibition of the growth of fungi and bacteria. Fresh aqueous extract of garlic showed antifungal activity specifically against some *Aspergillus spp*. including *A. fumigates, A. terreus, A. nidulans*, and *A. niger*. The inhibitory effects of allicin against *Trichophyton spp* are more pronounced than those of the essential oils derived from other plants. Garlic oil (GO) exhibits antifungal activity against *Candida albicans* and *Penicillium funiculosum* by penetration into cells and organelles and causing differential expression of genes that are critical for cellular metabolism (Li *et al*., 2014)

Anti-diabetic Properties

Diabetes affects a large segment of the population worldwide, and the prevalence of this disease is rapidly increasing. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, and higher life span. (Yajnik, 2001).

Despite the availability of medication for diabetes, traditional remedies are desirable and are currently being investigated. Many herbal medicines have been recommended for the treatment of diabetes (Marles and Farnsworth, 1995; Alarcon-Aguilara *et al*., 1998). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000). Garlic, which is a common cooking spice and has a long history as a folk remedy, has been reported to have antidiabetic activity. Garlic is a rich natural source of bioactive sulfur-containing compounds with potential antidiabetic properties

Evidence suggests that garlic's antioxidative, antiinflammatory, and antiglycative properties are responsible for garlic's role in preventing diabetes progression and the development of diabetes-related complications. Large-scale clinical studies with diabetic patients are warranted to confirm the usefulness of garlic in the treatment and prevention of diabetes. Preclinical research demonstrated that garlic’s active sulfur-containing compounds lowered hyperglycemia by enhancing the antioxidant capacity in diabetic rat circulatory systems. Furthermore, the garlic component functions as a donor of hydrogen sulfide, which regulates type 2 diabetes (Zhu *et al.,* 2018; Melino *et al.,* 2019). Human studies are also available where hypoglycemic effect of garlic was reported. The beneficial effects of garlic are mainly attributed to the presence of volatile sulfur compounds like alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene and allyl mercaptan. Garlic and garlic extracts have been shown to be effective in reducing insulin resistance. Therefore, considering the importance of garlic in controlling diabetic complications, several preparations and food processes containing garlic have been patented.

Anti-cancer Properties

The anti-cancer action of garlic is likely the best researched of the many advantageous pharmacological effects, and its use offers significant protection against the risk of developing cancer. A few active metabolites of garlic have been reported to be essential in the destruction of malignant cells due to their multi-targeted activities and lack of significant toxicity. The bioactive compounds in garlic having anticancer properties include diallyl trisulfide, allicin, allyl mercaptan diallyl disulfide, and diallyl sulphide. Different garlic-derived constituents and their nanoformulations have been tested for their effects against various cancers including skin, ovarian, prostate, gastric, breast, and lung, colorectal, liver, oral, and pancreatic cancer. It has also been demonstrated that garlic extract, its bioactive compounds, and their use in nanoformulations can prevent breast cancer in all of its stages, including initiation, promotion, and progression. The therapeutic and preventive properties of garlic against numerous cancers have been assessed by several epidemiologic, preclinical, and clinical investigations.

The anti-cancerous potential of garlic has been validated by many preclinical studies using human cancer cells, including those of the lung, mouth, stomach, pancreas, ovary, endometrium, breast, prostate, and bone cancer (Dorant *et al*., 1993; Tanaka *et al*., 2006). Further, laboratory experimentation has demonstrated that chemical components reported in garlic can repair DNA damage, induce cancer cell growth arrest, and reduce inflammatory responses. Consumption of garlic reduced the risk of esophagus, stomach, and colon cancer, according to human population research.

Mechanisms behind this growth suppression include the activation of metabolizing enzymes for carcinogen detoxification, inhibition of reactive oxygen species (ROS) generation and DNA adduct formation, control of the cell cycle, and apoptosis induction, prevention of angiogenesis,

invasion, and migration and thus reducing the anticancer agent's negative effects. It has been shown that the components of Garlic Oil which contain diallyl sulfide and diallyl disulfide compound prevented mutagenesis by blocking cytochrome P-450 2E1, which is required for the conversion of the cancer (Zhang *et al*., 2020). Reactions catalyzed by phase II detoxifying enzymes generally promote the elimination of drugs, toxins, and carcinogens from the body. Consequently, increasing the activity of phase II enzymes, such as glutathione S-transferases (GSTs) and NQO-1, may help prevent cancer by enhancing the elimination of potential carcinogens. In animal studies, oral administration of garlic preparations and organosulfur compounds was found to increase the expression and activity of phase II enzymes in a variety of tissues (Munday and Munday, 2001). For example, DADS protected rodent liver against carbon tetrachloride (CCl4; an environmental pollutant)-induced lipid peroxidation and cell necrosis by blocking CYP2E1-mediated CCL4 metabolic activation and by up-regulating Nrf2 downstream genes for NQO-1, HO-1, GCL, GST, and superoxide dismutase (SOD1).

In human breast cancer cells, DADS inhibited TNF-α-induced release of MCP-1, a chemokine that promotes tissue remodeling, angiogenesis, and metastasis. Aged garlic extract was also found to suppress in vitro angiogenesis by inhibiting endothelial cell proliferation, loss of adhesion, motility, and tube formation.

Anti-hypertensive Properties

Hypertension is a major risk factor of cardiovascular diseases (CVD), which are the predominant cause of morbidity and mortality worldwide. The treatment and control of hypertension are extremely important for the prevention of CVD and its related diseases. Generally, several types of drugs are recommended as first-line anti-hypertensive medications, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), dihydropyridine

calcium channel blockers and thiazide diuretics. When blood pressure cannot be controlled with one drug, treatment with multiple anti-hypertensive agents, such as combinations of ACE inhibitors or ARBs with dihydropyridine calcium channel blockers or thiazide diuretics is required. In spite of those well-established anti-hypertensive medications for high blood pressure, uncontrolled hypertension remains prevalent worldwide.

The antihypertensive action of garlic is mainly due to organosulfur compounds, which promote factors that relax endothelium and lowering blood pressure. Furthermore, garlic was found to be effective in preventing thrombosis and platelet adhesion or aggregation in people (Afzaal *et al*., 2021). A meta-analysis study (Karin 2020) found garlic supplements to reduce blood pressure in people with high blood pressure. Researchers linked this effect to a 16–40% reduced risk of experiencing cardiovascular events. The studies noted that the effect of garlic was similar to some blood pressure medications but with fewer side effects. Also, a review (Toshiaki 2019) notes that allicin in garlic may limit the production of angiotensin II, a hormone that increases blood pressure. It may also relax your blood vessels, allowing blood to flow more easily. The most consistent antihypertensive effects of garlic were observed in studies using aged garlic extract (AGE), another garlic preparation produced through natural aging for >10 months. AGE has been shown to significantly reduce blood pressure in patients with uncontrolled hypertension, suggesting that it may be used as a potent adjunct therapy for uncontrolled hypertension (Ried, 2013). Intriguingly, the study demonstrated that AGE lowered arterial stiffness, decreased inflammation and improved gut microbiota beneficially for cardiovascular health. AGE has also been shown to improve peripheral circulation in hypertensive rats, increase the plasma level of Nitric Oxide in mice and induce endothelium-dependent vasorelaxation of isolated rat aortic rings. An in vitro study has proven that garlic sulfur compounds, which are produced when red

blood cells convert the organic polysulfide of garlic to hydrogen sulfide, have vasoactive properties and are a recognized endogenous cardiovascular protective vascular cell-signaling molecule.

Anti-inflammatory properties

Garlic-derived organosulfur compounds have been found to inhibit mediators of the inflammatory response, including cytokines, chemokines, adhesion molecules, and enzymes like cyclooxygenase (COX), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) (Ho and Su, 2014). Nuclear factor-kappa B (NF-κB) is a transcription factor that binds DNA and induces the transcription of the COX-2 gene, other pro-inflammatory genes, as well as genes involved in cell proliferation, adhesion, survival, and differentiation. The anti-inflammatory effects of organosulfur compounds result from their ability to counteract the activation of pro-inflammatory pathways — like NF-κB-, MAPK-, and PI3K/Akt-dependent signaling pathways — by pro-inflammatory stimuli. DATS inhibited bacterial lipopolysaccharide (LPS)-induced macrophage activation by limiting LPS binding to toll-like receptor 4 (TLR4) and blocking the upregulation of TLR4 and TLR4-associated molecule MyoD88 expression. DATS also inhibited LPS-induced NF-κB-dependent expression of COX-2, iNOS, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β).

2.4. Methods of Application of Garlic

There are many consumable forms of garlic on the market, i.e., fresh garlic, garlic extract, garlic oil, dehydrated oil macerate, temperature aging garlic bulbs, and garlic powder. However, garlic is used for therapeutic purposes in tablet, oil, and powder forms. Garlic is also used in the form of juice, syrup, pills, and tincture (Labu and Rahman, 2019). Garlic supplements come in many

forms, including capsules, tablets, soft gels and those made from garlic powder. Some are deodorised, and others have an enteric coating to prevent 'garlic breath'. The processing steps for garlic have been confirmed to impact the final bioactive potency directly, and slight differences in the level of active ingredients may also cause profound variations in the bioavailability and final bioactivity of the final products. It is also advised that a combination of processing techniques should be preferred, depending on the expected functional properties.

Garlic oil which is mostly prepared by steam-distillation process. The Steam distillation of crushed garlic cloves results in a product that contains mainly allyl sulfides, including DATS, DADS, and DAS. These fat-soluble steam distillation products are usually dissolved in vegetable oil (Lawson and Bauer, 1998).

Aged garlic extract is another widely studied garlic preparation. Sliced draw garlic stored in 15-20% ethanol for more than 1.5 year is refereed to aged garlic extract. This whole process is supposed to cause considerable loss of allicin and increased activity of certain newer compounds, such as S-allylcysteine, sallylmercaptocysteine, allixin, N-0 - (Ideoxy- D-fructos-1-yl)-L-arginine, and selenium which are stable and significantly antioxidant

Aqueous Garlic extract: Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme is activated and produce allicin from alliin (present in intact garlic). Other important compounds present in garlic homogenate are 1 - propenyl allyl thiosulfonate, allyl methyl thiosulfonate, (EZ)-4, 5, 9 -trithiadodeca- l, 6, 11-triene 9- oxide (ajoene), and y-L-glutamyl-S-alkyl- L-cysteine. The adenosine concentration increases several-fold as the homogenate is incubated at room temperature for several hours.

Powdered or dehydrated garlic is made from garlic cloves that are usually sliced and dried at a low temperature to prevent alliinase inactivation. To meet United States Pharmacopeial Convention (USP) standards, powdered garlic supplements must contain no less than 0.1% γ-glutamyl-S-allyl-L-cysteine and no less than 0.3% alliin (dry weight). Although powdered garlic supplements do not actually contain allicin. Because alliinase is inactivated by the acidic pH of the stomach, most powdered garlic tablets are enteric-coated to keep them from dissolving before they reach the neutral pH of the small intestine.

Garlic oil macerate: Incubation of crushed garlic cloves in oil at room temperature results in the formation of vinyldithiins and ajoene from allicin, in addition to allyl sulfides, such as DADS and DATS.

Garlic tincture is a potent infusion of garlic in a solution of grain alcohol, vinegar or distilled water. The bulbs of the plant are used in this method of preparation. Garlic is macerated in one of said liquids, strained through cheesecloth into a glass jar and stored in a cool-dark location. There are a number of reasons why you may wish to choose garlic tincture over other delivery forms including fresh, capsules or tablets. The liquid used for the tincture (vodka for example) will extract the alcohol soluble components that are found within the garlic. While fresh garlic can spoil quite quickly, tinctures can be kept for up to a year when stored in glass jars in cool, dark settings. Tinctures are also believed to be more bioavailable to and more efficiently used within the body than the dried forms of the herb found in pills and capsules.

Plate 2.2: Aged Garlic Extract Bulb (Source: Hasib *et al*., 2016)

2.5. Clinical and Medicinal Benefits of Garlic

In current traditional medicine, garlic is used in Hemorrhoid treatment. Application of garlic gel on the skin may be beneficial in the treatment of alopecia areata (hair loss) in males and females. It has also found relevance in fighting bacteria and viruses. It is also useful in painful uterine disorder (endometriosis). Taking garlic powder tablets by mouth daily for 3 months seems to improve pain in people with this condition. It is also useful for people with a serious gum infection (periodontitis). Taking aged garlic extract by mouth twice daily for 18 months can help improve gum health in people who have mild or moderate periodontitis. Raw garlic has the potential to ward off cough and cold infections. Eating two crushed garlic cloves on an empty stomach has the maximum benefit. For kids and babies, hanging garlic cloves in a thread around their necks is supposed to relieve congestion symptoms.

Garlic promotes brain health because of its antioxidant and anti-inflammatory properties. It is effective against neurodegenerative diseases like Alzheimer's and dementia. Digestive problems

improve with the inclusion of raw garlic in the diet. It benefits the intestines and reduces inflammation. Eating raw garlic helps to clear out intestinal worms. The good thing is that it destroys the bad bacteria and protects the good bacteria in the gut. Garlic helps prevent acne and lightens acne scars. Cold sores, psoriasis, rashes, and blisters can all benefit from the application of garlic juice. It also protects against UV rays and therefore prevents ageing. Garlic reduces the expression of genes responsible for the formation of adipose cells which store fat. It also increases thermogenesis in the body and leads to the burning of more fat and the lowering of LDL (bad cholesterol). Apart from the fact that it is good for weight loss, garlic is highly nutritional. Garlic is considered one of the best" performance enhancing" substances. In olden times, the item was used to treat fatigue and improve the work capacity of labourers. Studies on rodents suggest consuming garlic helps in improving exercise performance. People who had heart disease consumed garlic for 6 weeks and this resulted in a 12% reduction in their heart rate and better exercise capacity.

According to studies from Japan, raw garlic when aged in a mixture of water and alcohol may have significant effects on exercise endurance. Human studies have also been conducted that have shown that garlic can indeed improve the symptoms of exercise fatigue. For people who are susceptible to lead poisoning due to occupational hazards, garlic may be the best organic solution. Studies conducted in 2012 have revealed that garlic is in fact, safer and better at reducing lead poisoning of the blood than d-Penicillamine, which is the common drug used to treat the same. The period of menopause for older women has often been associated with a lack of the female hormone known as oestrogen due to irregular production of a protein known as a cytokine. Consumption of garlic has been seen to regulate this to some extent and therefore, may be effective in overcoming oestrogen deficiency after menopause. Consuming garlic in your regular

diet can also help to prevent or reduce the onset of osteoarthritis. Research has shown that garlic contains a compound known as diallyl disulphide which helps to maintain bone density and therefore can potentially delay the onset of bone-related ailments like osteoarthritis. Garlic is also believed to help reduce the stickiness of the platelets in your blood. These platelets are responsible for the clotting of blood. Consuming a healthy dose of garlic can help reduce the excessive clotting effect of platelets on the blood. Therefore, it may help prevent unnecessary blood clots inside arteries that may reach up to your heart causing a heart attack.

Ear drops with mullein, St. John's wort, and garlic in an oil or glycerin base are traditional remedies used to alleviate symptoms, particularly pain, during acute ear infections. Garlic may help combat opportunistic infections. In one trial, an aged garlic extract reduced the number of infections and relieved diarrhea in a group of patients with AIDS. Garlic has been shown to kill parasites, including amoeba and hookworm, in test tubes and in animals. Other studies support the use of garlic to treat roundworm, pinworm, and hookworm. In one trial, patients with sickle cell anemia who were given folic acid plus aged garlic extract, vitamin C, and vitamin E saw significant improvement and less painful crises. The compound ajoene, found in garlic, is an antifungal agent that has been shown to be effective against athlete's foot. Crushed, raw garlic applied topically may also be effective. Based on preliminary research, topical use of garlic aqueous extract or lipid extract may beneficial in the treatment of warts and corns. Further study is needed.

2.6 Safety Evaluation with Garlic

An amount of garlic that is beneficial and safe for those who enjoy fresh garlic is eating 1 to 2 cloves daily. Garlic can also be in capsule form to avoid bad breath. The commonly suggested amount is 600 to 900 mg divided into 2 or 3 equal amount. For aged garlic extracts, a suggested

supplement is 3 to 7 g daily (Kharel, 2010). The majority of studies have stated that garlic supplements are very safe. The most commonly reported adverse effects of oral ingestion of garlic and garlic supplements are breath and body odor. Gastrointestinal symptoms have also been reported, including heartburn, abdominal pain, belching, nausea, vomiting, flatulence, constipation, and diarrhea. The most serious adverse effects associated with oral garlic supplementation are related to uncontrolled bleeding. Several cases of serious postoperative or spontaneous bleeding associated with garlic supplementation have been reported in the medical literature. Garlic may also trigger allergic responses in some individuals, including asthma in people with occupational exposure to garlic powder or dust. Exposure of the skin to garlic has been reported to cause contact dermatitis in some individuals. More serious skin lesions, including blisters and burns, have also been reported with topical exposure to garlic for six or more hours. The safety characteristics of the various garlic preparations likely depend on their specific chemical composition. Aged garlic extract the only water-based garlic supplement showed a safe profile in toxicity studies and exhibited no undesirable side effects when combined with anticoagulants (warfarin), antiplatelets (aspirin), cholesterol-lowering (statins) drugs, or anticancer drugs (doxorubicin, 5-fluorouracil, methotrexate) in clinical settings. Safety and toxicity data are lacking for lipophilic (hydrophobic) garlic preparations, but some of their constituents have been shown to interfere with drug-metabolizing enzymes and transporters.

Garlic is likely safe during pregnancy in amounts usually eaten in food, based on historical use. However, garlic supplements or large amounts of garlic should be avoided during pregnancy due to a possible increased risk of bleeding. In addition, early animal studies suggest that garlic may cause contraction of the uterus. Many tinctures contain high levels of alcohol, and should be avoided during pregnancy. Garlic is likely safe during breastfeeding in amounts usually eaten in

food, based on historical use. However, some mothers who take garlic supplements report increased nursing time, milk odor, and reduced feeding by the infant. The safety of garlic supplements during breastfeeding is not known.

2.7 TURMERIC

Curcuma longa or turmeric is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated extensively in Asia mostly in India and China. The rhizome, the portion of the plant used medicinally, yields a yellow powder. Dried *Curcuma longa* is the source of turmeric, the ingredient that gives curry powder its characteristic yellow color. It has many names such as Curcum in the Arab region, Indian saffron, Haridra (Sanskrit, Ayurvedic), Jianghuang (yellow ginger in Chinese), Kyoo or Ukon (Japanese). (Goel *et al*., 2008).

Turmeric has been used in Asian cuisines for both its flavor and color and in the Chinese and Ayurvedic medicine particularly as an anti-inflammatory and for the treatment of jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. It is official in the Pharmacopoeia of China as well as in other Asian countries such as Japan and Korea and its usage covers a wide range of health indications. In China it is ingested orally and applied topically for urticaria and skin allergy, viral hepatitis, inflammatory conditions of joints, sore throat and wounds.

Oral administration is the main route of administration for *Curcuma longa,* it can also be used topically and via inhalation (Ayurvedic tradition) or can be applied topically for the treatment of acne, wounds, boils, bruises, blistering, ulcers, eczema, insect bites, parasitic infections, hemorrhages and skin diseases like herpes zoster and pemphigus (Labban, 2014).

CHEMICAL COMPOSITION OF TURMERIC

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated7 (Figure 1). Curcumin was first isolated8 in 1815 and its chemical structure was determined by Roughley and Whiting9 in 1973. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform (Chattopadhyay *et al*., 2004).

2.8 ACTIVE CONSTITUENTS OF TURMERIC

The active constituents of turmeric are the flavonoid Curcuminoids which is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bisdesmethoxycurcumin Curcumin makes up approximately 90% of the curcuminoid content in turmeric. Other constituents include sugars, proteins, and resins. The bestresearched active constituent is curcumin, which comprises 0.3-5.4% of raw turmeric (Heath *et al*., 2004).

Turmeric is comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin (Figure 1), as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. The Curcumin is a lipophilic polyphenol that is nearly insoluble in water but is quite stable in the acidic pH of the stomach. The phenolic groups in the structure of curcumin explain the ability of curcumin to eliminate oxygen-derived free radicals. The free radicals which can be eliminated by curcumin are hydroxyl radical, singlet

oxygen, superoxide radical , nitrogen dioxide and NO (Labban, 2014). With regard to pharmacokinetic, studies have demonstrated that 40-85% of an oral dose of curcumin passes through the gastrointestinal tract unchanged. Due to its low rate of absorption, curcumin is often formulated with bromelain for increased absorption and enhanced anti-inflammatory effect. This review focuses on the medicinal and pharmacological properties of turmeric as anti-inflammatory, antioxidant, hepatoprotective, anticarcinogenic, antidiabetic, antimicrobial, antidepressant in addition to its use in cardiovascular disease, gastrointestinal and neurological disorders (Labban, 2014).

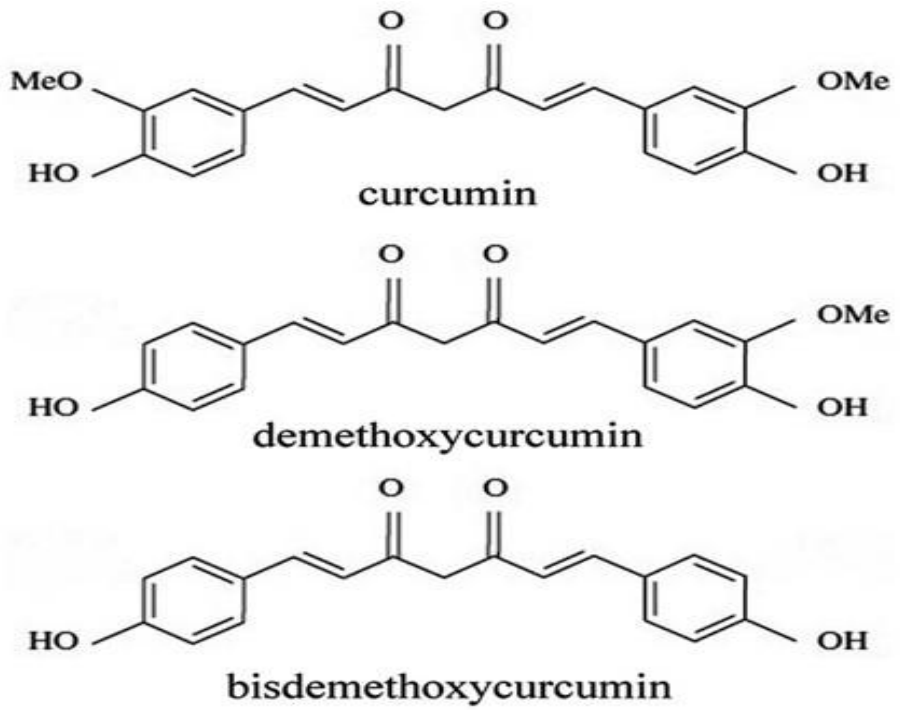

Figure 2.1: Structural formula of three curcuminoids ((Labban, 2014).

2.9 TAXONOMY OF TURMERIC

Turmeric was described as C. longa by Linnaeus and its taxonomic position is as follows:

Class Liliopsida

Subclass Commelinids

Order Zingiberales

Family Zingiberaceae

Genus Curcuma

Species Curcuma longa

The wild turmeric is called *C. aromatica* and the domestic species is called *C. longa.* (Chattopadhyay *et al*., 2004).

Plate 2.3: Turmeric rhizome and powder (Debjit *et al*., 2009).

2.10 PHARMACOLOGICAL BENEFITS/MECHANISM OF ACTION OF TURMERIC

Anti-inflammatory benefits

Oral administration of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone. Oral administration of Curcuma longa significantly reduced inflammatory swelling (Cronin, 2003). C. longa's anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states. Curcuminoids also inhibit LOX, COX, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon inducible protein, TNF and interleukin-12. They also decrease prostaglandin formation and inhibit leukotriene biosynthesis via the lipoxygenase pathway (Bundy *et al*., 2004). An RCT investigated the effect of a combination of 480mg curcumin and 20mg quercetin (per capsule) on delayed graft rejection (DGR) in 43 kidney transplant patients. Of 39 participants who completed the study, two of 14 in the control group experienced DGR compared to zero in either treatment group. Early function (significantly decreased serum creatinine 48 hours post-transplant) was achieved in 43% of subjects in the control group, 71% of those in the low dose treatment group. Since the amount of quercetin in the compound was minimal, the majority of benefit is thought to be due to curcumin's anti-inflammatory and antioxidant activity. Likely mechanisms for improved early function of transplanted kidneys include induction of the hemeoxygenase enzyme, and proinflammatory cytokines, and scavenging of free radicals associated with tissue damage (Shoskes *et al*., 2005).

Antifertility activity

Petroleum ether and aqueous extracts of turmeric rhizomes show 100% antifertility effect in rats when fed orally43. Implantation is completely inhibited by these extracts. Curcumin inhibits 5a-reductase, which converts testosterone to 5a-dihydrotestosterone, thereby inhibiting the growth of flank organs in hamster. Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive (Chattopadhyay *et al*., 2004).

Bacterial and Viral Infections

Test tube and animal studies suggest turmeric may kill bacteria and viruses. But researchers don't know whether it would work in people. The aqueous extract of turmeric rhizomes has antibacterial effect. Curcumin also prevents growth of Helicobacter pylori CagA+ strains in vitro. Both curcumin and the oil fraction suppress growth of several bacteria like Streptococcus,

Staphylococcus, Lactobacillus, etc. Curcumin has been shown to have antiviral activity (Araujo *et al*., 2001). It acts as an efficient inhibitor of Epstein-Barr virus (EBV). Most importantly, curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1 integrase needed for viral replication. It also inhibits UV light induced HIV gene expression127. Thus curcumin and its analogues may have the potential for novel drug development against HIV (Verma *et al*., 2018).

Cardiovascular disorders

The antioxidants in turmeric also prevent damage to cholesterol, thereby helping to protect against atherosclerosis. In fact, the ability of the antioxidants in turmeric to decrease free radicals is similar to that in vitamins C and E. Since the antioxidant activities of turmeric are not degraded by heat (unlike most vitamins), even using the spice in cooking provides benefits.

Animal studies show that curcumin lowers cholesterol and triglycerides, another fat that circulates in the blood stream and is a risk factor for cardiovascular disease. In a recent study of atherosclerosis, mice were fed a standard American diet, rich in refined carbohydrates and saturated fat, but low in fiber. Some of the mice, however, received this diet plus turmeric mixed in with their food. After four months on these diets, the mice that consumed the turmeric with their food had 20 percent less blockage of the arteries than the mice fed the diet without the turmeric. In another study, rabbits were fed turmeric plus a diet designed to cause atherosclerosis. Several risk factors for the disease were improved, including a decrease in cholesterol, triglycerides, and free-radical damage (Krup *et al*., 2013).

Anticarcinogenic properties

Animal research demonstrates inhibition at all three stages of carcinogenesis-initiation, promotion, and progression. During initiation and promotion, curcumin modulates transcription factors controlling phase I and II detoxification of carcinogens; down-regulates proinflammatory cytokines, free radical-activated transcription factors, and arachidonic acid metabolism vicyclooxygenase and lipoxygenase pathways; and scavenges free radicals. Studies involving rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. Turmeric and curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types in both in vitro and in vivo studies. (Labban, 2014). The anticarcinogenic properties of turmeric and curcumin are due to direct antioxidant and free-radical scavenging properties, as well as their ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation and Curcumin also induces apoptosis of cancer cells and it

inhibits angiogenesis. The efficacy of curcumin or turmeric extract in reducing chemically-induced tumours was studied by. Application of both curcumin and turmeric extract during carcinogenesis and promotion resulted in less papilloma production, compared to controls. This indicates that both curcumin and turmeric extract produce their best properties during tumour promotion. The effect of dietary curcumin (0.2% and 1.0%) on dimethylbenz (a) anthracene (DMBA) and tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumor formation was investigated by Limtrakul *et al*. (2001). They found a significant lower number of papillomas in the curcumin treated group compared to the control group. The enhanced expression of ras-p21 and fos-p62 oncogenes were decreased dose dependently in the curcumin treated group. The effect of Curcuma longa on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injury was investigated by Mohanty *et al*. (2006). Curcuma longa demonstrated significant anti-apoptotic property, which might contribute to the observed preservation in cardioprotective properties and cardiac function. The protective effect of turmeric extract on chemically induced mutagenicity in Salmonella typhimurium strains and clastogenicity in mammalian bone marrow in female Swiss mice (Labban, 2014). The anticarcinogenic properties were assessed in the benzo (a) pyrene induced forestomach neoplasia model. Aqueous turmeric extract exhibited antimutagenic activity against direct acting mutagens and also inhibited the mutagenicity of benzo (a) pyrene in Salmonella typhimurium strains. Treatment with the aqueous tumeric extract inhibited the development of forestomach tumors induced by benzo (a) pyrene significantly (Labban, 2014). These findings were all dose-dependent. There is some evidence that curcumin inhibits the activity of certain chemotherapy drugs. Research reveals curcumin decreased camptothecininduced death of cultured breast cancer cells and prevented cyclophosphamide-induced breast tumor regression in mice.

Curcumin might also interfere with the absorption and efficacy of the chemotherapy drug irinotecan, which is used to treat colon cancer. On the other hand, curcumin may enhance the effects of some chemotherapy drugs. In a mouse xenograft model of human breast cancer, curcumin in conjunction with paclitaxel (Taxol) significantly inhibited breast cancer metastasis to the lung to a greater degree than either curcumin or paclitaxel alone (Labban, 2014).

Antivenom effect

Ar-turmerone, isolated from C. longa, neutralizes both haemorrhagic activity of Bothrops venom and 70% lethal effect of Crotalus venom in mice4. It acts as an enzymatic inhibitor of venom enzymes with proteolytic activities (Chattopadhyay *et al*., 2004).

Indigestion

Curcumin stimulates the gallbladder to produce bile, which some people think may help improve digestion. The German Commission E, which determines which herbs can be safely prescribed in Germany, has approved turmeric for digestive problems. And one double-blind, placebo-controlled study found that turmeric reduced symptoms of bloating and gas in people suffering from indigestion. Turmeric powder has beneficial effect on the stomach. It increases mucin secretion in rabbits and may thus act as gastroprotectant against irritants. However, controversy exists regarding antiulcer activity of curcumin. Both antiulcer and ulcerogenic effects of curcumin have been reported but detailed studies are still lacking (Verma *et al*., 2018).

Hepatoprotective

The powder of the rhizome mixed with amla juice is used in jaundice. Corriliyum (Anjana) with Haridra, Red ochre (Gairika), and Amalaki (Emblica officinalis) cures jaundice. .Curcumin, the most common antioxidant constituent of Curcuma longa rhizome extract, was reported to

enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver (Krup *et al*., 2013). The ethanolic extract of Curcuma Longa rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg and 500 mg/kg, and the protective effect was dosedependent. The main constituents of Curcuma longa rhizome ethanolic extract are the flavonoid curcumin and various volatile oils, including tumerone, atlantone, and zingiberene. The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification. The volatile oils and curcumin of Curcuma longa exhibit potent antiinflammatory effects (Salama *et al*., 2013).

Antimicrobial properties

Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite Eimera maxima demonstrated that diets supplemented with turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain. Another study, in which guinea pigs were infected with eitherdermatophytes, pathogenic molds, or yeast, found that topically applied turmeric oil inhibited dermatophytes and pathogenic fungi. Improvements in lesions were observed in the dermatophyte- and fungi-infected guinea pigs, and at seven days post-turmeric application the lesions disappeared (Labban, 2014).Curcumin has also been found to have moderate activity against Plasmodium falciparum and Leishmania major organisms.

Khattak *et al*., (2005) studied the antifungal, antibacterial, phytotoxic, cytotoxic and insecticidal activity of an ethanolic extract of turmeric. The extract showed antifungal activity towards Trichophyton longifusus and Microsporum canis and weak antibacterial activity against

Staphylococcus aureus. Toxic activity was observed against Lemna minor. The Curcuma longa treated rabbit group showed a significant higher mean value for contraction of the wound compared to controls. Furthermore the wounds showed less inflammation and an increasing trend in the formation of collagen (Kundu *et al*., 2005).

Management of Obesity

People who would like to lose a couple of pounds or treat obesity and other similar condition can take benefits of turmeric powder which can be very helpful in keeping one's ideal body weight. The component in turmeric helps in boosting the flow of bile which is an essential element in the process of breaking down of dietary fats (Verma *et al*., 2018).

Neuroprotective activity

Curcuma oil significantly reduces the ill effect of ischemia by attenuating nitrosative and oxidative stress. Ischemia induces collapse of mitochondrial membrane potential, cytochrome c release, altering the Bax: Bcl-2 ratio and subsequently caspases activation led to induction of apoptosis in sequential fashion was reverse significantly by Curcuma oil. So there is an evidence for the high efficacy of Curcuma oil as a neuroprotective, with an excellent therapeutic window for the prevention of ischemic brain injury (Krup *et al*., 2013).

Dyspepsia and gastric ulcer

In a phase II clinical trial involving 45 subjects with endoscopically diagnosed peptic ulcers were given 600mg curcumin five times daily for 12 weeks. Ulcers were absent in 12 patients (48%) after four weeks, in 18 patients after eight weeks, and in 19 patients (76%) after 12 weeks (Labban, 2014). The remaining 20 patients, also given curcumin, had no detectable ulcerations at the start of the study, but were symptomatic-erosions, gastritis, and dyspepsia. Within 1-2 weeks

abdominal pain and other symptoms had decreased significantly. Kim *et al*., (2005) investigated the protective effect of turmeric ethanolic extract against gastric ulcers by blocking H2 histamine receptors (H2R) of male Sprague-Dawley (pylorus-ligated) rats. The effect of Curcuma longa extract was compared to the properties of ranitidine. Curcuma was found to protect the gastric mucosal layer as effective as ranitidine. Orally administerd ethanolic extract inhibited gastric acid, gastric juice secretion and ulcer formation comparable to the properties of ranitidine. The antiulcer activity of an ethanolic extract of turmeric investigated. Administration of turmeric extract led to a significant decrease in ulcer index and acidity of stomach contents. Pretreatment with the turmeric extract reduced the intensity of ulceration. Hypothermic-restraint stress reduction of gastric wall mucus was inhibited by turmeric extract treatment and reduced the severity of lesions induced by various necrotizing agents (Labban, 2014).

Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are the two primary forms of inflammatory bowel disease (IBD). Holt *et al*., (2005) conducted a pilot study to examine the effect of curcumin therapy in patients with IBD who had previously received standard UC or CD therapy. Hematological and biochemical blood analysis, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) (the latter two inflammatory indicators), sigmoidoscopy, and biopsy were all performed at baseline and at the study end (Labban, 2014). Crohn's Disease Activity Index (CDAI), CRP, ESR, hematological blood analysis, and kidney function was assessed in all patients at baseline and end of study. In the proctitis group all five patients improved by study's end as indicated by a global score, and all five subjects demonstrated normal ESR, CRP, and serologic indices of inflammation after two months. In the CD group, CDAI scores decreased by an average of 55 points, and CRP and ESR decreased in four of five patients. The authors

concluded that curcumin plus standard therapy was more effective in maintaining remission than placebo plus standard UC treatment (Hanai *et al*., 2006).

2.11 SOME REMEDIES OF TURMERIC

Anemia

Everyday take a dose of 1 tsp of turmeric juice mixed with honey.

Asthma

Boil 1 cup of milk with 1 tsp of turmeric powder. Drink warm.

Burns

Mix 1 tsp of turmeric with 1 tsp of aloe gel and apply to burnt area.

Conjunctivitis

Mix 1 tbsp of crushed, raw turmeric in 1/3 cup of water. Boil and sieve. 2–3 drops of this mixture may be used in each eye up to 3 times per day.

Complexion

Apply a paste of turmeric on the skin before bed, and wash off after a few minutes. In the morning, remove any remaining yellow tinge with a paste of chickpea flour (besan) and oil.

Dental problems

Mix 1 tsp of turmeric with ½ tsp of salt. Add mustard oil to make a paste. Rub the teeth and gums with this paste twice daily.

Diabetes

½–1 tsp of turmeric should be taken 3 times a day. (Debjit *et al*., 2009).

PHARMACOKINETIC STUDIES ON CURCUMIN

Curcumin, when given orally or intraperitoneally to rats, is mostly egested in the faeces and only a little in the urine. Only traces of curcumin are found in the blood from the heart, liver and kidney. Curcumin, when added to isolated hepatocytes, is quickly metabolized and the major biliary metabolites are glucuronides of tetrahydrocurcumin and hexahydrocurcumin. Curcumin, after metabolism in the liver, is mainly excreted through bile (Chattopadhyay *et al*., 2004).

2.12 CLINICAL STUDIES AND MEDICINAL APPLICATIONS OF TURMERIC AND CURCUMIN

Although various studies have been carried out with turmeric extracts and some of its ingredients in several animal models, only a few clinical studies are reported so far.

Turmeric

Powdered rhizome is used to treat wounds, bruises, inflamed joints and sprains in Nepal. In current traditional Indian medicine, it is used for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Data are also available showing that the powder, when applied as capsules to patients with respiratory disease, gives relief from symptoms like dyspnoea, cough and sputum. A short clinical trial in 18 patients with definite rheumatoid arthritis showed significant improvement in morning stiffness and joint swelling after two weeks of therapy with oral doses of 120 mg/ day. Application of the powder in

combination with other plant products is also reported for purification of blood and for menstrual and abdominal problems (Chattopadhyay *et al*., 2004).

Curcumin

In patients undergoing surgery, oral application of curcumin reduces post-operative inflammation. Recently, curcumin has been formulated as slow-release biodegradable microspheres for the treatment of inflammation in arthritic rats. It is evident from the study that curcumin biodegradable microspheres could be successfully employed for therapeutic management of inflammation (Chattopadhyay *et al*., 2004).

2.13 SAFETY EVALUATION WITH TURMERIC AND CURCUMIN

Detailed studies have been reported on the safety evaluation of the rhizomes of C. longa and its alcohol extract, curcumin. The major findings are presented below.

Turmeric

The average intake of turmeric by Asians varies from 0.5 to 1.5 g/day/person, which produces no toxic symptoms.

Male and female Wistar rats, guinea pigs and monkeys were fed with turmeric at much higher doses (2.5 g/kg body wt) than normally consumed by humans. No changes were observed in the appearance and weight of kidney, liver and heart. Also, no pathological or behavioural abnormalities were noticed and no mortality was observed (Chattopadhyay *et al*., 2004).

Curcumin

Curcumin was given to Wistar rats, guinea pigs and monkeys of both sexes at a dose of 300 mg/kg body wt. No pathological, behavioural abnormalities or lethality was observed. No

adverse effects were observed on both growth and the level of erythrocytes, leucocytes, blood constituents such as haemoglobin, total serum protein, alkaline phosphatase, etc. Human clinical trials also indicate that curcumin has no toxicity when administered at doses of 1–8 g/day and 10 g/day (Chattopadhyay *et al*., 2004)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Laboratory Animals

Adult female healthy albino rats weighing between 140–200 g were acquired from the experimental animal house of Pharmacology and Toxicology Department, Faculty of Life Sciences, University of Benin, Benin City. The animals were acclimatized for seven (7) day before starting the experiment and were maintained on standard pellet diet and tap water and kept at 25 ± 3^0C temperature, 50–60% humidity, and a 12 h light-dark cycle.

3.2 Plant Material Collection and Extraction

The Turmeric and garlic were bought from Ekosodin market, in Ugbowo, Benin City, Edo state in December, 2023. The fresh Turmeric and garlic were washed and placed on a chopping board, with the crown and the bottom of the fruit resting on its base. The two plants were respectively cut into pieces. The diced turmeric and garlic were separately dried at room temperature for two weeks, then it was transferred to the oven at a temperature of 45^0 C. Upon drying, the plants were pulverized into powder and weight, transferred into air tight jar. 1000 g of Turmeric and garlic was weight each and transferred into a jar 2.5 litters of N-hexane solvent was poured into the glass jar and scooped for some minutes after which the sample contained in the jar was tight closed, wrapped with black cloth .the sample was transferred to a dark cupboard for 73 hours after which the samples were filtered with cheese cloth to separate the fiber from the extract .the extracted sample was dried over a water bath, the emanating powder from the bi-herbal formulation was weighed and the yield was calculated.

3.3 Acute Toxicity Study

Acute toxicity study was carried out by methods of OECD (Organization of Economic Co-operation Development), 2008a guidelines. Six (6) female mice were administered 5, 000 mg/kg per body weight of the extract and observed for 72 hours for possible signs of toxicity, mortality or morbidity.

3.4 Animal Model

Twenty five female albino rats (weighing between 180 – 200 g) were obtained from the experimental animal house in our institute and were used for the study. The rats were housed in wire mesh cages under standard conditions (temperature 25 -29 C, 12h light and 12h darkness cycles). Animals were fed with pelleted standard rat diet (Top feeds ltd, Nigeria) and water. Generally, the study was conducted in accordance with the standard guard principles in care and use of animals.

3.5 Experimental Design

The twenty (25) female albino rats were randomly categorized into six experimental groups of five (5) animals each:

Group A: (Control) - administered feed and water only

Group B: 50 mg/kg/rat extract + feed and water

Group C: 250 mg/kg/rat extract + feed and water

Group D: 500 mg/kg/rat extract + feed and water

Group E: 10 mg/kg/rat Tamoxifen + feed and water

Group F: 10 mg/kg/rat Diethystibesterol + feed and water

Daily oral dose of the extract was administered to each animal in the different groups using orogastric cannula, for a period of 28 consecutive days.

3.6 Body and Reproductive Organ Weight

Body weight of control and experimental animals were weighed at the beginning of the study and on the day of sacrifice. 24 hours after the last dose of the extract, the rats were sacrificed by cervical dislocation. The uterus was dissected out, and cleared of fat and connective tissue. The uterus was removed and weighed.

3.7 Hormonal Assay

After the animals were sacrificed 4ml of blood was collected by cardiac puncture. The blood sample was centrifuged at 2500 rpm for 10 min and serum used for the progesterone (P), luteinizing hormone (LH) and follicle stimulating hormone (FSH) assay by the enzyme linked immunoassay technique.

Statistical Analysis

Data are expressed as mean ± SEM and analyzed using the student's test and ANOVA where necessary. $P \leq 0.05$ was accepted as significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Study

Acute toxicity study revealed that there was no sign of toxicity, mortality or morbidity after administration of 5000 mg/kg per body weight of the extract and observed for 72 hours (Table 4.1).

4.2 Effect of the N-hexane extract of Bi-herbal mixture of Turmeric and garlic on reproductive organ weight

The oral administration of doses of 50, 100 and 250 mg/kg of the N-hexane extract Bi-herbal mixture of Turmeric and garlic (**NETUGA)** on Female rats for 28 days resulted to significant decreases in reproductive organ weight. The result revealed that the product is an anti-fertility agent for the females.

4.3 Effect of N-hexane extract of Bi-herbal mixture of Turmeric and garlic on Reproductive Hormonal Assay in the Sera of Female Wistar Rats

Female Wistar rats weighing 180-200 g administered 50, 250 and 500 mg/kg showed significant decrease in reproductive hormones such as Estrogen 2 (E2) mean while Leuthenizing hormone (LH), follicle stimulating hormone (FSH) were revealed to be reduced significantly after 28 days treatment and were significant at with P-value at * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$. (Table 4.2).

The oral administration of doses of 50 and 250 and 500 mg/kg of the N-hexane extract Bi-herbal mixture of Turmeric and garlic (**NETUGA)** on Female rats for 28 days resulted to significant

decreases in Estrogen 2 (EST 2) with significant reduction in leuthenizing hormone (LH) and follicle stimulating hormones. The result revealed that the product is an anti-fertility agent for the female counterpart in their early reproductive ages as estrogen 2 (EST 2) is present in only women of child bearing ages

CHAPTER FIVE

5.0 DISCUSSION

5.1 Acute Toxicity Study

In the acute toxicity studies, no death was recorded during the treatment period at all doses of the garlic bulb extract administered. The animals were apparently healthy with no sign of toxicity up to the dose of 5000 mg/kg. Thus, LD_{50} was more than 5000mg/kg (Bashir Lawal *et al*., 2015).

5.2 Body and Reproductive Organ Weight

Oral administration of N-hexane bi herbal mixture of turmeric and garlic on female Wistar rats showed significant variation in the reproductive organ, weight of experimental animals in the study group. The result obtained revealed decreased reproductive organ mass after administering 50, 250 and 500 mg/kg of (NETUGA) extracts for 28 days. After 28 days treatment with 50, 250, and 500 mg/kg of the (NETUGA) extracts the result obtained also revealed a significant decrease in female reproductive organ uterus. When compared with the control and was similar with the result of tamoxifen treated female rat which was observed to have presented with reduction in reproduction organ mass as was expected since tamoxifen is an antifertility drug used in prevention of pregnancy as shown in (Figure 4.1). Furthermore, there was a significant ($p \leq 0.05$) difference in Female Reproductive Organ (FRO) mass when compared to control in a dose-dependent manner. The result obtained evidently proves, that the bi-herbal mixture used may possess some oestrogen- like activity (Yusuh *et al*., 2010; Radenahmah *et al*., 2011).

5.3 REPRODUCTIVE HORMONAL LEVELS

The result obtained from this research showed significant decrease in reproductive hormone such as estrogen 2 (E2) mean while leuthenizing hormone (LH), follicle stimulating hormone (FSH), were revealed to be reduced significantly as showed in (Table 4.2). This explains that this product (NETUGA) is not only a antifertility agent for the female counterpart but it is positively involved in a longer term infertility of male counterparts in their early reproductive ages as estrogen 2 (EST2) is present in only women of child bearing ages.

The female hormone responsible for reproduction is estrogen. Follicle stimulating hormone (FSH) and the luteinizing hormone (LH) releases the ovaries in female that makes up estrogen. Estradiol (E2), Estriol (E3) and Estrone (E1) are the natural estrogen in females. Including E2 there are several important sex hormone produced by women more during their reproductive year. For E3 after the period of menopause, begins during pregnancy while for the fourth stage of estrogen, estetrol (E4) begins. Estrogen would further binds to estrogen receptors (ERS) which is situated in the nucleus of the cell membrane (Ghosh, 2018).

CONCLUSION /RECOMMENDATIONS

The present study has proven that the N-hexane extracts of bi- herbal mixture of turmeric and garlic on experimental rats revealed that the product is not only antifertility agent for the female counterpart but it is positively involved in long-term fertility of male counterparts in their early reproductive ages as estrogen 2 (E2) is present in only women of child bearing age. It causes a decrement in the reproductive hormonal level such as estrogen 2 (E2) meanwhile LH, FSH were revealed to be reduced significantly. There was also a decreased reproductive organ mass present in the treated female wistar rat. This indicates that there was no occurrence of reproductive enzyme dysfunction leading to harmful effect of the reproductive tract. This current research is currently the initial step in the scientific documentation of N-hexane extract of bi- herbal mixture of turmeric and garlic on female reproductive organs in laboratory animals. A comprehensive study is required to support the above assertions and demonstrate the health benefits of the mixture of this formulation (turmeric and garlic extract).

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