

**IMMUNOMODULATORY EFFECT OF COLD PRESSED COCONUT OIL  
EXTRACT ON WISTAR RAT**

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BENIN CITY**

**DECEMBER, 2019**

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**B.Sc (Crop Science) Benin**

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**DECEMBER, 2019**

## CERTIFICATION

This is to certify that the project work was carried out by Deborah Osasere IGORI (Miss) of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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**EXTERNAL EXAMINER**

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**DATE**

## **DEDICATION**

This project work is dedicated to Our Lord Jesus Christ, the author and finisher of my life, for His mercy, His graces and innumerable favor upon my life. I say thank you Lord

## ACKNOWLEDGEMENTS

I thank God Almighty for His strength to do this work. My heartfelt gratitude goes to my parents Mr. and Mrs. Osagie Austin Igori for their financial and moral support. Mr. Philip, Nehemiah, Abigail, Mr. and Mrs Gbenro for their love and care, you are all highly appreciated.

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I also want to appreciate my friends. I love you all

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## ABSTRACT

Coconut oil has a unique role in human nutrition, its clinical studies reveal an effect on human health. Herbal plants are traditionally used in folklore medicine for various diseases. This work aimed at investigating the immunoprotective effects of cold-pressed coconut oil in immunosuppressed Wistar rat. A standard procedure was used to carry out immunomodulatory properties of the extract with modifications. The immunomodulatory properties of coconut oil were investigated by testing the immune responses to the antigen challenges with sheep red blood cells (SRBCs). The animals were placed into six groups of five rats per group. Coconut oil was given orally at the dosage level of 0.25, 0.5 and 1.0ml/kg for 21 days; also 0.3ml i.p of SRBCs. Evaluation was carried out by testing the hematological parameters. The result obtained showed a significant increase in 0.25ml dose after twenty-one (21) days. White blood cell ( $11.70 \pm 1.25$ ) had same value with control and HGB ( $15.57 \pm 0.67$ ), increase significantly when compare with control. The CD4 and CD8 had better result with coconut oil when compared with the control. The Anti-Oxidant activities had a positive response in 0.25ml. Histological result reveals a healthy heart, lungs, spleen, liver and kidney in lower dose of oil extract. In conclusion, Coconut oil can be said to contain immunomodulatory effect in accordance with folklore report.

## CHAPTER ONE

### INTRODUCTION

The immune structure is extremely multipurpose defence scheme that has transcended to guard flora and fauna from invading pathogenic microbes and to eradicate disease, which can generate an vast variety of cells and molecules competent of recognizing and eliminating actually limitless variety of foreign invaders (Richard *et al.*,2003). Immunomodulation is required when host defence system has to be active below the circumstances of impaired immune response or when a selective immunosuppressant is implicated in situations like autoimmune disorders. Medicinal plants are comparatively a recent aspect in phytomedicine, which deals extensively with herbal materials in the formulations of supplements. Immunomodulatory management using medicinal plants which serves as an alternative approach to conventional medicine against several diseases, when host defence method is activated under the situation of impaired immune response. The immune structure is known to be concerned in the investigation and pathologic mechanisms of numerous diseases, which has greatly increased the necessitate of drugs which are effectual on immune structure. To triumph over the delay in the artificial immunomodulators, there is a need of improvement of herbal immunomodulators (Z-ziza *et al.*, 2008).

Immunomodulatoris substances either natural or synthetic, which kindle and modulate any of the mechanism of the immune system including innate and adaptive arms of the immune reaction (Agarwal and Singh, 1999). Immunomodulation is the regulation and response of immunity either by enhancing or by falling the immune defence. Modulation of an immune response may involve initiation, expression of immune response caused by agents that activate or suppress its function (Shivaprasad *et al.*, 2006). If the modulation in the immune structure results in augmentation of immune reaction, it is known as the immunostimulator. The modulation of the immune system

by using herbal plant products as a potential therapeutic evaluation has become a subject of systematic investigations. Immunomodulation could offer an alternative to conventional chemotherapy for a diversity of diseased conditions, particularly when the host's defence mechanism has to be activated under the conditions of impaired immune reaction or when a choosy immunosuppression has to be induced in situations like auto-immune disorders and organ transplantation (Upadhyay, 1997).

Herbal supplements are known to acquire immunomodulatory ingredient and stimulate both specific and non-specific protection (Singh *et al.*, 2011). Some plants used in herbal medicine are reported to have immunomodulatory properties; some of these motivate humoral and cell-mediated immunity while others activate merely the cellular mechanism of the immune system, not including affecting the humoral or cell-mediated immunity. An organism that causes a disease is called a pathogen and the person or animal that is infected by a pathogen is called the host. There are numerous components to the immune structure and it seems that the immune structure is far more complex than required for achieving what is, on the exterior, uncomplicated task of eradicating a pathogenic organism (Parkin and Cohen, 2001). Nevertheless, the reasons for this complication including the interest of eliminating pathogens without causing harm to the host. Destroying a pathogen is hypothetically easy, but eliminating these without detrimental effect to the host is much more complex. The deduction of this active complexity of the immune scheme to build a large variety of cells and molecules that is able of specifically recognizing and destroying an apparently vast diversity of foreign invaders, moreover the recognition and obliteration of irregular cells (Parkin and Cohen, 2001). The necessary task of the immune system is to guard the organism against contagious agents and pathogens, which puts the immune system in an important place between the healthy and unhealthy state of a host. Its

defensive duty is regulated by a health regulatory mechanism linking both humoral and cellular factors. The immunomodulatory component can augment the immunological reaction of an organism by interfering with its processing mechanisms that may openly induce production of mediators and effectors molecules by the immunocompetent cells. The side effects connected with synthetic drugs approve plants to be safer, cheaper, and much more effective, conventional immunomodulator plants can be investigated (Diasio *et al.*, 1996).

The coconut palm (*Cocos nucifera* L.) family *Arecaceae* and subfamily *Cocoidae* is a humid tree species which formerly was the first main estate crop, extending over huge uniform geographical areas, but is currently largely grown and harvested by minute farmers (Assa *et al.*, 2010). It is one of the mainly significant rainforest trees grown in over 90 countries in the globe. Coconut palm is called the "tree of life" since the extensive range of possibilities for its uses (Assa *et al.*, 2010). In addition to the manufacture of vegetable oil for commercial uses, health, bio-fuel, cosmetic, and wellness products, it is also a food, fiber and beverage crop, and also an illustration amenity palm for, city parks, golf courses, village gardens, also for tourist purpose throughout the tropics. From its natural territory on the shoreline of unpopulated oceanic islands to inland locations on the outer edge of deserts and the foothills of rock ranges, it can supply every necessity for the continued existence of castaways, subsistence utilization, local and global markets. Physicians and Health care professionals have display an unwillingness to use coconut oil as a health food. Saturated fats have been neglected for so several years, that they find it difficult to change their ideas even when faced with confirmation to the contrary.

Oil is the mainly well-known food manufactured goods got from dehydrated coconut pulp, called the copra (Shankar *et al.*, 2013). The various method used to extract fat from the coconut, can be differentiate from the final product. Virgin oil (unrefined oil), is gotten from mature and fresh

coconuts by pressing procedure, the oil got in this process does not contain chemical compound and other bleaching properties (Villarino *et al.*, 2007) which also maintain its natural properties and a high content of bioactive compounds. (Arunima *et al.*, 2013). Refined oil, is gotten by subjecting the coconut oil to further processing like refining, deodorizing and bleaching, because of these procedure, result in a tasteless and odourless oil product. (Marina *et al.*, 2009) coconut oil gotten by fermentation technique, pure cultures of probiotic bacteria are used to extract oil from the coconut milk, which resulted in volatility of emulsion, the oil phase pile up at the top of the surface and demarcate from other fractions such as water, carbohydrates and proteins, the oil gotten by this technique is yellow and has a characteristic fermented aroma (Handayani *et al.*, 2009). Coconut oil in cooking raises many controversies, mostly because of the elevated content of saturated fatty acids (90%) usually regarded as damaging to human health. Nevertheless, there are several clinical studies that recommend its positive effect on the growth of the organism and on the protection of the existing functions, both adults and children (Shankar *et al.*, 2013).

## **1.1 AIM OF THE STUDY**

The goal of the study is to examine the immunomodulatory activity of cold-pressed coconut oil extract in male wistar albino rats.

## **1.2 SPECIFIC OBJECTIVES**

The deficiency of the immune system by a foreign invader is still a serious health challenging issue according to recent publications. It is against this background this research sought to investigate the immunomodulatory effect of *Cocos nucifer* oil extract in the management of an infectious disease.

The goals of the study are to test;

1. To estimate the hematological parameter activities of the plant oil extract
2. To estimate the CD4 and CD8 Count
3. Investigate the liver function test activities of the plant oil extract
4. To evaluate the antioxidant and immunomodulatory properties of the plant oil extract
5. To evaluate the histological result of the plant oil extract on Wistar rat.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 THE INFLUENCE OF COCONUT OIL ON HUMAN NUTRITION

Cancer diseases have an enormous collision on the society of the world. The definition of cancer means the uncontrolled increase of changed cells, which leads to teaching of malignancy, and also reduces the enlargement of normal cells in the human being (Tan *et al.*, 2010).

The significance of coconut oil supplement for patients with cancer of the breast. The study was evaluated with sixty women ( $\geq 18$  years) in three or four breast cancer, and also they had six cycles of chemotherapy Law *et al.* (2014). Patients (n=30) received twice a day 10 ml of unprocessed coconut oil while 3, 4, 5 and 6 cycles of chemotherapy (different to the control group). The coconut oil serves as supplement which enhanced the general state of the patients and has also assist to diminish the unwanted effects of chemotherapy.

The study was to ascertain the effect of coconut oil on cancer cells situated in the lungs (in vitro studies with human being cells at a concentration of  $1 \times 10^5$ ). The results established the ability of unrefined coconut oil to slow down the enlargement of cells (effects recorded with coconut oil at a dose of 10% during 72 hr incubation) and induction of their death (added coconut oil at a concentration of 8.64 and 12.04% during 72 hr incubation). However, coconut oil is believed to contribute in the increasing cholesterol substance in the blood, due to the presence of high saturated fats (Kamalaldin *et al.*, 2015).

In several clinical studies, there is no relationship between the utilization of virgin coconut oil and the improvement of coronary heart disease in populace placed on diet containing significant quantity of coconut oil (Lipoeto *et al.*, 2004).

There is a misunderstanding spreading among numerous individual that coconut oil is not excellent for heart wellbeing, because it contains a large amount of saturated fats. General, saturated fats contains about 50% lauric acid, which assist in active prevention of various heart problems like high blood pressure and increase cholesterol levels. The presence of saturated fats in coconut oil is not detrimental which is commonly found in vegetable oils, also having antimicrobial properties and facilitate in production with various parasites, bacteria and fungi that can cause dyspepsia. Coconut oil does not lead to enhance (LDL) levels, and it reduces the damage to arteries and occurrence of wound, therefore aid in preventing atherosclerosis (Vala *et al.*, 2014).

Coconut oil is extremely functional for weight loss. It contains medium and small-chain fatty acids that assist in taking off unnecessary weight gain. It is also easy to absorb, and it aid in healthy performance of the endocrine and thyroid system. Additional, it improve the body's metabolic charge by eliminating stress on the pancreases; thus burning extra energy and helping overweight and obese individual lose their weight. Hence people existing in humid areas, who use coconut oil each day as their main cooking oil, are normally not obese and fat.

Coconut oil aid in improving the digestive structure and therefore prevents diverse stomach and digestion associated problems including Irritated bowel syndrome. It enhances assimilation of other nutrients such as amino acids, vitamins, and minerals. Lauric acid found in coconut oil plays a significant role in preventing various cardiovascular diseases such as high blood pressure. (Assunção *et al.*, 2009) there was a comparison between soybean oil (a supply of unsaturated fatty acids) and coconut oil on the lipid profile of 40 female's blood (20-40 years old). The study comprises of a daily consumption of 30 ml of coconut or soybean oil for 12 weeks. The lipid

profile of females who used up coconut oil was categorized with a decrease LDL and increase in HDL cholesterol level.

Various studies shown with the human involvement, both male and female, have established the nutritional properties of coconut oil. This product decreases not only the overall level of body overweight but also speeds up the breakdown of body fat Tsuji *et al.* (2001) investigation with the healthy persons involvement has established that diet rich in MCTs has weight lost element and assist to reduce body fat. Other writers have examined the effect of MCTs on persons with increase triglyceride levels in serum. Also reducing body fat, the percentage of triglycerides reduced by 15% (Xue *et al.*, 2009).

Wound therapeutic is a complex procedure where the body tissue heals itself after damage. The oil of *Cocos nucifera* has been stated to have wound healing properties. (Nevin *et al.*, 2010) considered the result of topical claim of virgin coconut oil (VCO) on skin mechanisms and also the antioxidant level of dermal infections healing in baby rats. Another study shows that animals were administered for 10 days with VCO, a day after formation of the wound. VCO's healing measure was assessed by monitoring time for comprehensive epithelization besides several features of the wound's granulation tissue. Animal model given VCO displayed much faster-wound curative activity, presented by a decreased in time of complete epithelization and increased levels of several dermis component. Summarily, wound healing movement of VCO may be a collective effect of several minor biologically active mechanisms present (Nevin *et al.*, 2010).



**Plate 1:** Coconut and the extracted oil

## **2.2 IMMUNOMODULATORY INFLUENCE OF *Cocos nucifera***

In 1966, Jon Kabara studied the Medium-Chain Fatty Acids (MCFA's) of virgin coconut oil, and its antimicrobial properties that kill dangerous fungi, viruses, parasites and bacteria. When MCFA's are assimilated, they are broken down into free fatty acids and monoglycerides (Ogbolu *et al.*, 2007). Capric, Caprylic, and Lauric Acid, are the significant medium-chain fatty acids existing in coconut oil that possess antimicrobial movement. Their monoglyceride forms include, monolaurin, monocaprin and monocaprylin obstruct microbes from invading the immune structure. Separately, these fatty acids act on microorganisms in different methods. Some may murder a particular organism that causes fungal contaminations but may not be as valuable on other microbes. Nevertheless, they act as extremely powerful defence against sicknesses. Monolaurin is known for having the best antifungal, antiviral, and antibacterial influence (Shilling *et al.*, 2013). Another study demonstrated that, as associated to other oils, coconut oil in the diet boost insulin action and enhanced binding affinity (Ginsberg *et al.*, 1982).

## **2.3 INFLUENCE OF COCONUT OIL ON COSMETICS**

Coconut oil is outstanding massage oil for the skin. It perform an effective conditioner on all skin type. The value of coconut oil on the skin is correspondent to that of mineral oil. Auspiciously, unlike mineral oil, there is no occurrence of having any adversarial side effects on the skin from the use of coconut oil. Coconut oil is a nontoxic solution for inhibiting dryness and peeling of skin. It also delays the occurrence of wrinkles and sagging of skin which normally supplementing ageing. Coconut oil also helps in handling various skin problems as well as psoriasis, eczema, dermatitis and additional skin problems. For that particular reason, coconut oil forms the base requirements of several body care products like lotions, creams, and soaps that

are used for skincare. Coconut oil also helps in averting premature ageing and deteriorating disease because of its well-known antioxidant possessions.

Coconut oil is one of the finest natural nutrients for hair. It aids in a healthy development of hair shiny value. It is also very active in reducing protein loss which can lead to various unpleasant or unhealthy growth potentials in your hair. Majority of the people in developing countries apply coconut oil on their hair every day after showering. It is a tremendous conditioner and helps the re-growth development of damaged hair. It also offers the vital proteins required for nurturing and healing broken hair. Research show that, coconut oil offers better protection to hair from loss caused by hygral fatigue. By frequently massaging your head using coconut oil, make sure that your scalp is free of dandruff; even your scalp is extremely dry. It also helps in protecting your hair and scalp free from lice eggs and lice. Coconut oil is therefore used as hair care lubricant. Coconut oil is applied topically for hair care daily growth.

## **2.4 INFECTION AND HEALING**

Coconut oil forms a chemical coating that defends the infected body part from exterior air, dust, fungi, viruses and bacteria when applied to infected part of the body. Coconut oil is extremely active on bruises since it speeds up the healing procedure of damaged tissues. Coconut oil is very active against a diversity of infections because of its antiviral, antibacterial, and antifungal, properties. Coconut oil kills the viruses that cause, measles, hepatitis, influenza, herpes, and other serious health risks. It also destroys bacteria that cause gonorrhoea, throat infections, pneumonia, urinary tract infections, and ulcers. Coconut oil is active in the eradication of yeasts and fungi infections that cause diaper rash, athlete's foot, ringworm, and thrush.

## 2.5 ECONOMIC UTILIZATION AND NUTRITIONAL VALUE OF COCONUT OIL

Trunks and Leaves serves as construction materials, and roots has healing properties. The most vital part of the plant is a fruit in which we can distinguish the outer layer (husk), used in the manufacture of carpets, ropes and fibers, and the inner layer which is processed into cosmetic products and various food items—coconut water, coconut milk, coconut shavings, coconut flour and coconut oil (endosperm) (Prades *et al.*, 2012). Coconut oil is the most famous food product gotten from dehydrated coconut pulp.

The technique used in extracting fat from coconut can distinguish by different final product: Virgin oil (unrefined oil), got from fresh and mature coconuts by pressing process, the oil got in this method does not comprise of chemical solvents and other bleaching agents (Shankar *et al.*, 2013).

The composition of fatty acids includes saturated and unsaturated fatty acids (Uau *et al.*, 2006). Generally saturated fatty acids have a harmful influence on human wellbeing, mostly because of their effects on the lipid level in blood serum. They support platelet accumulation and thus intensify the threat of thrombus development in blood vessels. Regardless of many opposing effects on the human health, they are excellent source of vitality. Humans react differently to unsaturated fatty acids effects, which is characterized by at least a single dual bond in the structural chain particle. These acids are accountable for the growth in the level of HDL fraction (High-Density Lipoproteins), they also result in a reduction in the level of LDL cholesterol in blood serum (Szponar *et al.*, 2008).

Coconut oil belongs to a unique class of oil products called lauric oils, which contain high content of saturated fatty acids (90%), especially the contribution of lauric acid (C12: 0) Yousefi

*et al.* (2013). MCTs are the class of saturated lipids (three fatty acid molecules are linked directly to the glycerol skeleton) and they contain in their structural chain from 6 to 12 of carbon atoms. Coconut oil is a source of Medium-chain triglycerides (MCTs) Krishna *et al.* (2010).

Phytosterols are operational and functional analogues of saturated fat that is synthesized in the plant. It has hypocholesterolemic and anti-tumour effects (Batta *et al.*, 2006). They reduce the level of entire cholesterol and LDL fraction, and also stop platelet aggregation (Kopeć *et al.*, 2011). The entire content of sterols in coconut oil is about 70 mg/100 g (Verleyena *et al.*, 2002)

Vitamin E plays a major part as a natural antioxidant in food because of its protective possessions against unsaturated fatty acids decreasing the vulnerability of these components to unfavorable oxidation procedures (Adam *et al.*, 2009). Tocols are a basic block of cell membranes; they take part in many metabolic procedures and inhibit the ageing of the human body (Zielińska *et al.*, 2014).

Polyphenols are a class of chemical mixtures that are classified as secondary metabolites of plants (Pandey *et al.*, 2009). Its function is the ability to deactivate reactive forms of nitrogen and oxygen, and free lipid and protein radicals (Strumiłło *et al.*, 2015). Summarily, they possess a bactericidal, anti-allergic and anti-inflammatory properties, also they shield the skin from oxidative injury and prevent diabetes, cancer and cardiovascular diseases (Wilczyńska *et al.*, 2009).

## **2.6 IMMUNE STRUCTURE DEVELOPMENT**

Similarly, systems organ include the respiratory or circulatory system, the immune structure is vital for human life. The organs of the immune structure manage our interaction with the surroundings, forming defensive obstacles where needed, and offer both passive and active

defence against possible invaders. The complication of the human immune structure discloses an evolutionary arms contest of adaptation and counter variation which arose up to 4 billion years ago with the unassuming biochemical defences of unicellular organisms. The species-level development of the immune system determined by genetic changes is reproduced in a microcosm by the ontogenic development of each person's separate immunity. Ontogenic immune growth occurs through epigenetic variations both in utero and all through life. Even before reproduction, sperm use effective immunetolerising approaches to escape the female immune structure and allow zygote development; the mother's immune system obligation is to improve tolerance mechanisms to the alloantigen in the subsequent fetus and zygote. Throughout and after birth, the baby will be unprotected to a host of antigens over a quick period, the environment of which will disturb immune-structure development. Type of birth, include vaginal against caesarean, changes the introduction to microbiota, altering the immune structure; the immune system also prejudiced by the baby's emerging microbiome which has a solid influence on immune system growth (Aagaard *et al.*, 2016). The direct result of colostrum on immunity in the infant, breastfeeding also alter the baby's microbiota. Breastfed offspring have an additional diverse gut microbiome, which assist both immune improvement and homeostasis. This homeostatic result of the gut microbiome stays throughout the lifecycle, making conservation of diversity and prevention of deleterious factors, such as unwarranted antibiotic usage, a significant factor in maintaining a healthy immune structure. Epigenetic deviations continue throughout childhood with the immune structure reaching its useful peak in undeveloped adults. The main function-altering proceedings of immune structure include the above-mentioned reproductive immunosenescence, and immunotolerance. Immunotolerance involves ageing procedure of the immune structure. Immunosenescence intensify the danger of infection and loss of control of inflammatory

procedures, which may influence heart disease, Alzheimer's disease, stroke, and particular forms of neoplasia. Additionally, autoimmunity grows with age, accompanied by extra danger of disorders such as diabetes, myasthenia gravis, and rheumatoid arthritis. To place this risk into context, the lifetime risks of cancer and autoimmune disease in advanced countries are estimated at 30% and 10%, respectively. The function and structure of the immune system are solely the repair of tissue and disposal of damaged or neoplastic cells are also essential roles of the immune system. The growth of the gut microbiome, which enhances proper immune function, is a further crucial task. These distinct roles are illustrated by the healing of tissue following immune-related inflammation or the removal of cells infected by viruses, and the harmonious action of these characters is central to proper immune function. Like many systems and organs, the prevailing function of the immune system is adapting to sustain homeostasis in a constantly changing surroundings. This adaptive character is likely to be the reason behind the protection of the manifold immunological approaches advanced during the evolutionary development.

Constitutive immunity is stimulus-independent, containing mainly of obstacles, while innate and adaptive immunity is stimulus-dependent reactions. The innate system is characterized by a rapid but non-specific response, while the adaptive system performs gradually but with a specific and more effective response that grant long-term immunity. Constitutive immunity comprises of the natural AMP, skin, antimicrobial enzymes like lysozyme and mucociliary transport. Innate immunity involves mast cells, innate lymphoid cells (ILC), complement, basophils neutrophils, and eosinophils. The main effectors of the adaptive immune system are the multiple T and B lymphocyte subtypes, the latter of which yield Ig. The immune system functions are

overlapping and interdependent. Innate and adaptive immunity must operate in unison with the various detector and effector cells operating harmonically.

The production of secretion by goblet cells and the organized movement of cilia ease the mucociliary transport arrangement, further impeding the entrance of allergens and pathogens and aiding in their exclusion. Adequate humidity, precise temperature, and appropriate viscosity of the mucus are all significant factors for the purpose of the mucociliary transport method. AMP is anti-infective proteins formed at the constitutive barriers and is energetic against both Gram-negative bacteria and Gram-positive; in addition, AMP outbreak the viral envelope, protozoa fungi, and even cancer cells. AMP also has an immunomodulatory part, creating a chemotactic result, helping to decrease inflammatory reaction and support its resolution, and interacting with and controlling the adaptive immune reaction. AMP also aids to regulate the microbiota and act by two principal mechanisms: the formation of transmembrane pores, which has a cytolytic influence, and the diffusion of the cell membrane and disruption of vital processes through binding intracellular particles.

## **2.7 IMMUNE MECHANISM OF IMMUNOMODULATION**

Immunity can be defined as the body's capability to recognize and counterattack enormous numbers of infectious and possibly detrimental microorganisms, supporting the body to avoid or resist diseases and constrain tissue and organ injuries (Arshad *et al.*, 2013). The immune structure is not restricted to any one part of the body. Immune stem cells, which develop in the bone marrow, could linger in the bone marrow pending maturation or transfer to different body locations for development. Consequently, majority of the immune cells flow throughout the body, employing specific effects. The immune structure has two separate but overlapping means

through which to combat overrunning organisms, the antibody-mediated defence structure (humoral immunity) and the cell-mediated defence structure (cellular immunity). Ultimately, two dissimilar lymphoid cells, T and B cells which convey 'cellular' and 'humoral' immunity correspondingly. Mutually cells exist in the circulating blood and in peripheral lymphoid tissues. The acknowledgments of the antigen by the T cells indicate the multiplying of these cells, penetration of immune cells at the site of action and cellular immunity. The responses may be demonstrated as Delayed type Hypersensitivity, tissue graft elimination (Millind *et al.*, 2005). The other branch of the immune structure involving B cells is accountable for the origin of specific antibodies immunoglobulins (IgA, IgD, IgE, IgM). The acknowledgment of antigen (Ag) by the B cells leads to the propagation of these cells, change to plasma cells and succession of specific antibodies (Ab). The exact Ab binds with the specific antigen leading to its phagocytosis or inactivation. Phagocytic Activity by Nitrobluetetrazolium dye decline assay, Lymphocyte Propagation by mitogen (Agarwal *et al.*, 1999).

Immune system defends against attacking microorganisms, foreign cells and toxins. Immunodeficiency such as Digeorge's syndrome because of absence of T cells (lacking cellular immunity), agammaglobinaemia because of absence of B cells (deficient humoral immunity) and Severe combined immunodeficiency disease (SCID) is because of adenosine deaminase insufficiency causing death to B and T cells.

Drugs can moderate immune mechanism by either overpowering or by motivating one or more of the following steps:

1. Antigen acknowledgment and Phagocytosis
2. Lymphocyte proliferation/distinguishing
3. Formation of antibodies

4. Antigen-antibody association
5. Discharge of mediators for immune reaction
6. Alteration of target tissue reaction/target effector organ
7. Complement arrangement

Types of immune reaction occurring in the human body:

- Innate immune response
- Adaptive immune response

The innate immune reaction is the first line of defence operation against biochemical, cellular and physical components. The Adaptive immune reaction (Humoral immunity) Antibody production – eradicating extracellular organisms. Cellular immunity articulated as cytotoxicity towards target cells by triggering of cytotoxic or “killer” T-cells. The action of the cytotoxic T-cell inhibited by adrenocortico steroids<sup>1</sup>. But the humoral arm of the immune response is responsible for the production of the antibodies; carried out by cells resulting from the bone marrow ( $\beta$ -cell). Innate Immunity Activation of PRR is a main stage in starting the innate immune reaction. There are numerous types of PRR. TLR identify products known as pathogen-associated molecular patterns (PAMP) and create a rapid reaction, where B cells occupy the germinal centre of lymphoid follicles stimulated by follicular Th cells. Activated B cells multiply, often separating into plasmablasts that produce IgM for a quick but weak affinity Ig response. It is vital to note that the effector and memory phases of immunity are not limited to T and B lymphocytes. These stages are reflected in the regulatory cell classes that prevent extreme immune activity, with memory controlling cells persevering long-term and awaiting activation by antigens alongside memory T and B cells.

## 2.8 ROLES OF THE IMMUNE REACTION

The immune system recognizes and responds to antigens. To defend the individual efficiently against disease, the following tasks need to be considered:

1. Immunological acknowledgement: existence of an infection must be discovered (by the innate and adaptive immune systems)
2. Contain the infection and likely eliminate it, through several immune effector functions (e.g. antibodies, complement)
3. Immune regulation limits damage to the host by the immune response to antigen. Termination of this regulation contributes to disease conditions including autoimmune disease and allergy.
4. The adaptive immune system generates immunological memory—exposure to an infectious agent produces an immune response that can persist and protect the host in a subsequent exposure

The fundamental architecture of the immune structure is multifaceted, with defences on some levels. Most clear and principal is the skin: the main obstacle against infection. Additional physiological conditions like the pH of the body and temperature provide unfitting living situations for foreign organisms. When pathogens have effectively entered the body, they are faced by the innate or adaptive immune organism (Dinesh *et al.*, 2012). Both organisms comprise a mass of cells and particles that interact in a multifaceted manner to notice and eradicate pathogens (Rohini *et al.*, 2011). Discovery and eradication depend upon chemical bonding: tops of immune system cells enclosed with many receptors, some of which chemically bind to pathogens, however others fix to other immune structure cells or molecules to allow the compound signaling system that mediates the immune reaction (Janssen, 2001). Synthetic or

Biological constituents that can modulate or suppress, stimulate any feature of the immune structure including both adaptive and innate arms of the immune system (Khyati, 2012). The Arrangement of immunomodulators clinically, can be categorized into the following three categories: Immunoadjuvant embrace the promise of being the exact modulators of the immune reaction. Immunoadjuvant are used to improve the efficiency of vaccines and consequently could be considered exact immune stimulants. Immunostimulant are fundamentally nonspecific as they are visualized as improvements to a body's resistance to contamination. They can act through innate and adaptive immune reactions. In healthy persons, the Immunostimulant are predictable to serve as a prophylactic and stimulate agents, i.e., as immunopotentiator, by augmenting the basic stage of immune reaction (Arshad *et al.*, 2013). Individual with damage immune reaction, they are predictable to act as immunotherapeutic agents. Immunosuppressants are functionally and structurally heterogeneous group of drugs, which are often concurrently given in combination routines to treat many types of organ uproot rejection and autoimmune diseases.

## **2.9 DRUGS USED FOR IMMUNOMODULATION**

All drugs which modification immune response characterized as immunomodulators, which function as: Immunosuppressants, immunostimulant. Some of these can have together the properties depending on which element of immune reaction they affect. There is also future generation of immunosuppressants called tolerogen. Immunosuppressant drugs inhibit lymphocyte gene expression to lower inflammatory response glucocorticoids

## **CHAPTER THREE**

### **MATERIALS AND METHOD**

#### **3.1 PLANT MATERIALS**

In cold method extraction, coconut milk is permitted to wait for 20-24 hrs. Under favorable environments (35-40°C, 75% Relative Humidity), the oil gets separated from the water and the protein. The airborne lactic acid bacteria, which can breakdown the protein bonds, act on the coconut milk combination producing the VCO disassociation. It requires appropriate operating settings and hygienic precautions were strictly observed. Four different layers could be apparent in the vessel after settling for 20-24 hrs. The base layer was made up of sticky residue. The next layer was the watery, skim milk that is no longer appropriate for human ingestion. The next layer was the separated oil for retrieval as VCO. The top layer was moving curd. The curd also holds a significant quantity of trapped oil. By cautiously separating the distinctive layers, the oil can be separated. The separated oil contained some adhering particles of curd and it need to be filtered using sterilized filter paper placed in a large funnel for easy flow.

#### **3.2 ANIMALS MODEL**

Wistar albino male rats weighing between 150-315g, a total of thirty (30) respectively were gotten from the Animal House, Faculty of Life Sciences, University of Benin, Benin City. Wistar rats were accommodated under hygienic environments in the departmental animal house. These animals were given water and standard feeds during the experimental period (a month and sixteen days). They were acclimatized for seven days.

### **3.3 EXPERIMENTAL DESIGN**

The animals were weighed, numbered and then separated into six groups with five animals in each:

Group A: Rats served as a positive control fed with feed and water for 21 days

Group B: Rats were treated intra-peritoneally with SRBC for every 7 days

Group C: Rats were treated orally with 0.5ml/kg standard drug (Azathioprine) for 21 days

Group D: Rats were treated orally with 0.25ml/kg of coconut oil extract for 21 days

Group E: Rats were treated orally with 0.5ml/kg of coconut oil extract for 21 days

Group F: Rats were treated orally with 1.0ml/kg of coconut oil extract for 21 days

### **3.4 PREPARATION OF SHEEP RED BLOOD CELLS (SRBC)**

Fresh blood was obtained from a healthy sheep, sacrificed in the local slaughter house in a sterile bottle containing Alsever's solution (1:1). Then it centrifuged at 500g for 15 min at 4 C in cooling centrifugation apparatus to separate the red blood cells (RBCs). The Sheep red blood cell (SRBC) was washed three times in normal saline. It was then kept under refrigeration for the immunization.

### **3.5 ASSESSMENT OF THE IMMUNOMODULATORY ACTION OF CEVCO ON EXPERIMENTAL ANIMALS**

Thirty Wistar rat was used to evaluate the result of coconut oil on the immune structure. The rat was divided into six groups of five rat per cage.

The first group were given clean water and standard feed. This served as a positive control group (Group-A).

The second group served as a negative control group, were given clean water and standard feed. On the 7<sup>th</sup> and 14<sup>th</sup> days of the experiments, rat immunized with sheep red blood cell 0.3ml intraperitoneally (Group-B).

The third group served as the standard drug, orally administered 0.5ml dose of Azathioprine daily for 21 days and were given clean water and standard feed. On the 7<sup>th</sup> and 14<sup>th</sup> days of the experiments, rat where immunized with sheep red blood cell 0.3ml intraperitoneally (Group- C).

The fourth group was administered 0.25ml of CEVCO orally treated for 21 days and was given standard feed and clean water. On the 7<sup>th</sup> and 14<sup>th</sup> days of the experiments, rat was immunized with sheep red blood cell 0.3ml intraperitoneally (Group-D).

The fifth group was administered 0.05ml of CEVCO orally treated for 21 days and were given standard feed and clean water. On the 7<sup>th</sup> and 14<sup>th</sup> days of the experiments, rat where immunized with sheep red blood cell 0.3ml intraperitoneally (Group-E).

The sixth group was administered 1.0ml CEVCO orally treated for 21 days and was given standard feed and clean water. On the 7<sup>th</sup> and 14<sup>th</sup> days of the experiments, rat where immunized with sheep red blood cell 0.3ml intraperitoneally (Group-F).

### **3.6 COLLECTION OF SERUM SAMPLE**

At the end of the experiment, the animals were sacrificed and blood samples were collected. The serum was separated for various biochemical tests.

### **3.7 BIOCHEMICAL INVESTIGATIONS**

Liver Function Tests. The estimation of Alkanine aminotransferase (ALT), Aspartate aminotransferase (ALP), Alkanine phosphatase (ALP), Gama glutamine transferase (GGT), Total bilirubin (TOT. BIL), Conjugated bilirubin (CONG. BIL), Unconjugated bilirubin (UB) and Total protein (TP)

Anti-Oxidant Test. The estimation of Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Glutathione peroxidase (GPx)

### **3.8 HEMATOLOGICAL INVESTIGATION**

In completion of the experiment, rats were sacrificed under chloroform anaesthesia. Blood was collected through cardiac pinhole. The estimation of various (20) parameters was carried out. The White blood cell (WBC), Hemoglobin (HGB), Red blood cell (RBC), Platelet count (PLT) and lymphocytes.

### **3.9 STATISTICAL ANALYSIS**

The data were calculated as mean  $\pm$  Standard Error of Mean. Significance differences of the mean value with respect to the control group were examined by one way ANOVA using GraphPad Prism 8 Statistical Software.  $P < 0.05$  considered being significant

## CHAPTER FOUR

### RESULTS

The entire Wistar rats were apparently healthy throughout the period of study and no rat sustained any injury during the entire period of study. Though immunomodulatory properties of plants and animals origin improve the immune reaction of the body against pathogens by actuating mainly the non-specific immune structure. Throughout the study, several hematological, morphometric, serological, and other factors required to assess the immune status of the Wistar rats upon administration of *Cocos nucifera* oil extract for 21 days were recorded.

In this current study, the extract was administered orally at 0.25ml, 0.5ml and 1.0ml to group D, group E and group F, respectively. To test the effect of *cocos nucifera* oil extract on immune response, its influence was tested on SRBC induced in rats. From the results obtained in the present study, a significant increase ( $p < 0.05$ ) was observed at a dose of 0.25ml (group D) compared to an untreated control group (A). The results on the immunomodulatory studies conclude that, the albino rats administered with varying concentration of oil extract yield a positive result. One of the earliest immune response can be seen and measured by studying the haematological parameters such as leukocyte, white blood cell, red blood cell, granulocyte, lymphocyte count and other parameters were measured for the entire group. Blood cells are the main cells to react to invading non-self-materials.

**Table 1:** Hematological parameter of albino rats treated with coconut oil extract

| <b>Parameters</b> | <b>Control</b> | <b>Negative control</b> | <b>(5 ml/kg) Azathioprine</b> | <b>0.25 ml/kg coconut oil</b> | <b>0.5 ml/kg coconut oil</b> | <b>1.0 ml/kg coconut oil</b> |
|-------------------|----------------|-------------------------|-------------------------------|-------------------------------|------------------------------|------------------------------|
| <b>WBC</b>        | 11.73±1.51     | 10.10±0.17              | 9.30±0.45                     | 11.70±1.25                    | 9.90±0.76                    | 10.33±1.56                   |
| <b>LYM%</b>       | 83.07±3.53     | 84.43±0.59              | 84.47±1.68                    | 84.03±2.36                    | 83.10±1.10                   | 85.17±1.82                   |
| <b>MON%</b>       | 9.533±1.82     | 8.67±0.24               | 8.20±0.60                     | 8.83±0.93                     | 8.90±0.47                    | 8.10±1.00                    |
| <b>GRAN%</b>      | 7.40±1.70      | 6.90±0.50               | 7.33±1.13                     | 7.13±1.42                     | 8.0±0.67                     | 6.73±0.83                    |
| <b>LYM#</b>       | 9.77±1.39      | 8.53±0.18               | 7.87±0.41                     | 9.90±1.34                     | 8.20±0.50                    | 8.80±1.36                    |
| <b>MON#</b>       | 1.10±0.26      | 0.87±0.03               | 0.77±0.07                     | 1.00±0.00                     | 0.90±0.10                    | 0.83±0.14                    |
| <b>GRAN#</b>      | 0.87±0.23      | 0.70±0.00               | 0.67±0.12                     | 0.80±0.10                     | 0.80±0.15                    | 0.70±0.11                    |
| <b>RBC</b>        | 6.36±0.33      | 6.48±0.44               | 6.0±0.15                      | 6.85±0.25                     | 6.71±0.38                    | 6.37±0.27                    |
| <b>HGB</b>        | 13.60±0.53     | 14.63±0.89              | 13.83±0.50                    | 15.57±0.67                    | 15.33±0.70                   | 14.23±0.62                   |
| <b>HCT</b>        | 37.50±1.47     | 40.83±2.24              | 38.73±1.31                    | 42.37±1.48                    | 41.40±1.68                   | 38.70±1.11                   |
| <b>MCV</b>        | 59.13±0.84     | 63.20±1.60              | 64.77±0.64                    | 61.93±0.09                    | 62.57±0.37                   | 60.97±1.70                   |
| <b>MCH</b>        | 21.37±0.34     | 22.57±0.48              | 23.03±0.35                    | 22.67±0.14                    | 22.83±0.35                   | 22.30±0.10                   |
| <b>MCHC</b>       | 36.20±0.55     | 35.77±0.23              | 35.67±0.60                    | 36.67±0.29                    | 37.00±0.44                   | 36.73±1.17                   |
| <b>RDW-SD</b>     | 30.93±1.23     | 32.20±0.60              | 33.43±1.07                    | 31.57±1.07                    | 31.60±0.00                   | 32.83±3.26                   |
| <b>RDW-CV</b>     | 16.07±0.42     | 15.80±0.00              | 16.13±0.43                    | 15.70±0.52                    | 15.73±0.24                   | 16.50±1.27                   |
| <b>PLT</b>        | 740.00±72.76   | 765.30±45.33            | 949.70±322.00                 | 660.30±80.85                  | 909.00±132.30                | 996.30±225.90                |
| <b>MPV</b>        | 7.43±0.12      | 8.03±0.26               | 8.47±0.09                     | 7.77±0.09                     | 8.07±0.13                    | 8.20±0.30                    |
| <b>PDW</b>        | 9.37±0.33      | 10.47±0.70              | 11.17±0.44                    | 9.57±0.07                     | 10.40±0.59                   | 10.80±0.67                   |
| <b>PCT</b>        | 0.54±0.047     | 0.61±0.02               | 0.80±0.28                     | 0.51±0.06                     | 0.73±0.11                    | 0.82±0.21                    |
| <b>P-LCR</b>      | 3.83±1.93      | 8.30±3.30               | 12.80±1.12                    | 5.03±0.27                     | 8.03±2.43                    | 12.10±3.16                   |

The value are expressed as (Mean ± SD) n=5 per group p < 0.05.

**WBC** – White blood cell

**GRAN%** -Granulocyte

**P-LCR** –Platelet large cell ratio

**MCV** –Mean corpuscular volume

**MCHC** –Mean corpuscular haemoglobin conc.

**RDW-SD** –Red blood cell distribution width

**RDW-CV** - Red blood cell distribution width percentage

**MCH** –Mean corpuscular hemoglobin

**PDW** – Platelet distribution width

**MPV** –Mean platelet volume

**LYM%** - Lymphocyte

**LYM#** - Lymphocyte

**RBC** – Red blood cell

**HCT** – Hematocrit

**MON#** - Monocyte

**PLT** –Platelet count

**MON%** - Monocyte

**HGB** – Hemoglobin

**PCT** –Plateletcrit

**GRAN#** - Granulocyte

Group A: Rats served as a positive control

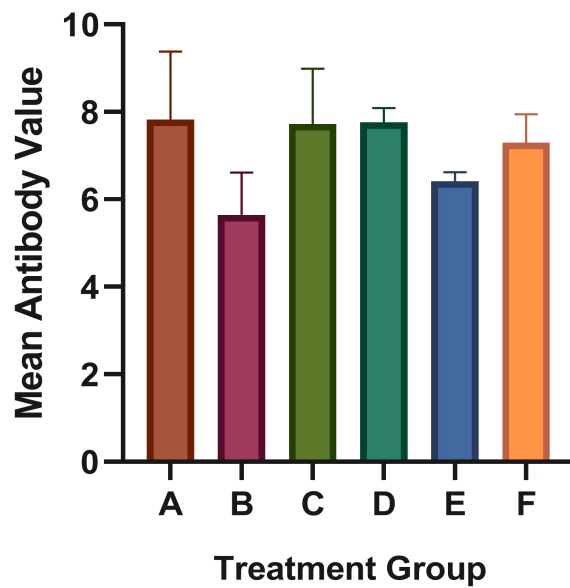
Group B: Rats were treated intraperitoneally with SRBC

Group C: Rats were treated orally with 0.5ml/kg standard drug (Azathioprine)

Group D: Rats were treated orally with 0.25ml/kg of coconut oil extract

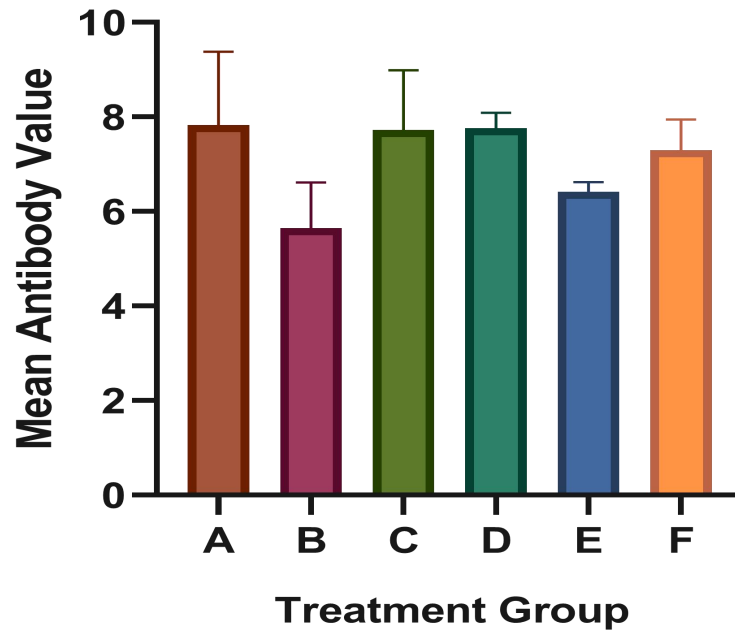
Group E: Rats were treated orally with 0.5ml/kg of coconut oil extract

Group F: Rats were treated orally with 1.0ml/kg of coconut oil extract



**Fig. 1: Effect of Coconut oil extract on CD 8 Count**

Shows an increase in the antibody level of the experimental group administered with 0.25ml, 1.0ml extract when compared with normal control. The extract was able to increase the CD8 Count in the affected rat when compared to an untreated group B, having the lowest mean value

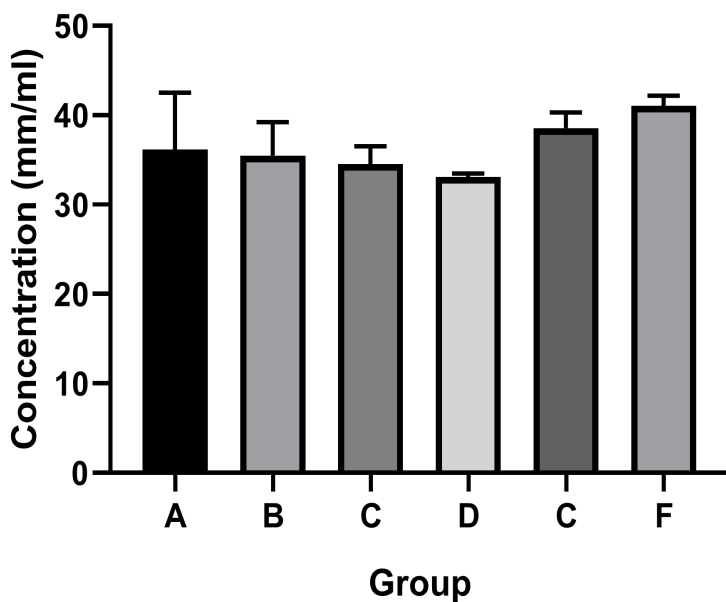


**Fig. 2: Effect of Coconut oil extract on CD 4 Count**

The results showed that high and low dose of *Cocos nucifera* oil extract-treated animals increase the CD4 Count level significantly when compared to control. Group D,  $7.763 \pm 0.185$ , group B  $5.643 \pm 0.558$ , group A  $7.830 \pm 0.894$ . The SRBC reduced the antibody response significantly when compared to the control group.

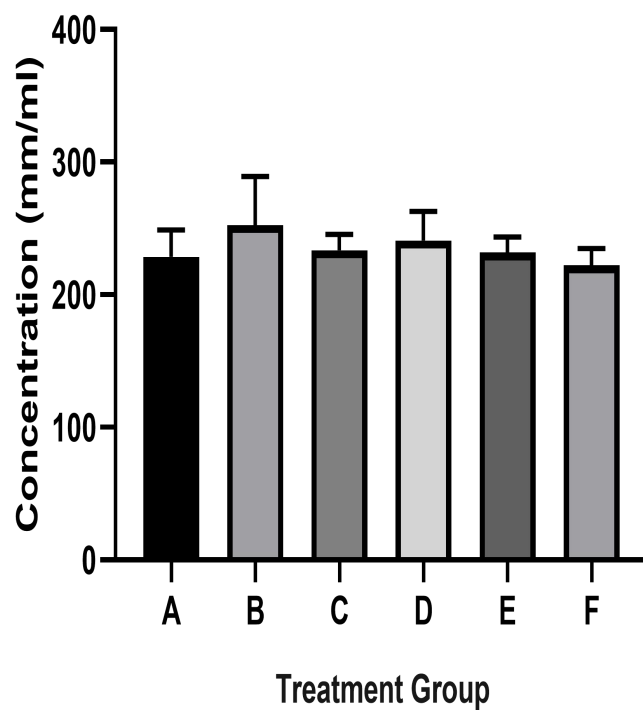
#### 4.1 EFFECT OF EXTRACT ON BIOCHEMICAL PARAMETERS

Anti-oxidant test carried out were to estimate the Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Glutathione peroxidase (GPx) responses in the cold press coconut oil extract in Wistar rat.



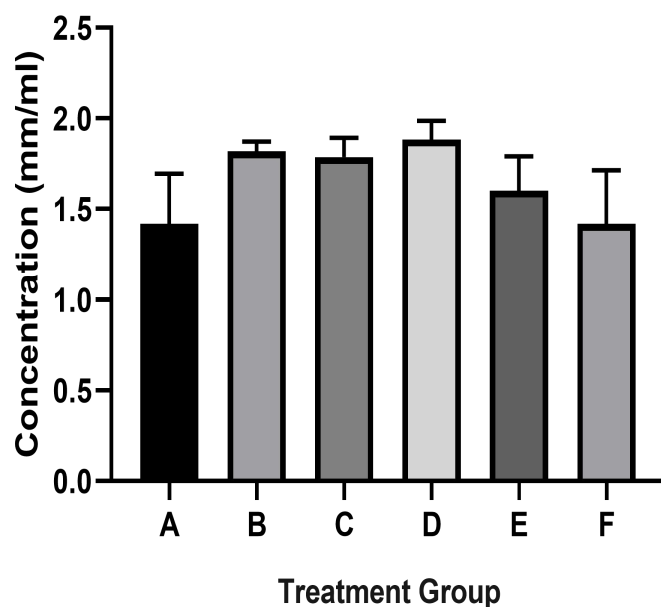
**Fig. 3: Effect of *Cocos nucifera* on Malondialdehyde Response**

Fig. 3 above reveals the effect of the extract on Malondialdehyde response on rat treated with a higher dose of extract 1.0ml/kg produced a significant increase of  $41.08 \pm 0.629$  against  $36.14 \pm 3.681$  control group. A lower dose of extract reduces the mean level.



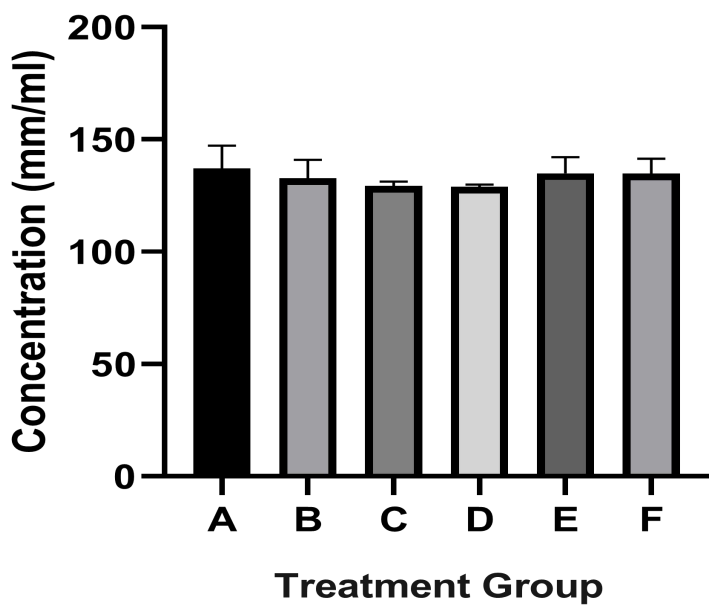
**Fig. 4: Effect of Extract on Catalase Response**

The effects of various doses of *Cocos nucifera* oil extract on catalase response in Wistar rat, compared with the control group.  $P < 0.05$  considered being no significant difference among the treatment group B having the highest catalase mean value.



**Fig. 5: Effect of Extract on Superoxide Dismutase Response**

Among the rats of group D and E which received extract treatment at concentration 0.25ml and 0.5ml respectively showed high antibody titre against control. Group F which received the highest extract dose (1.0ml) shows low antibody titre.

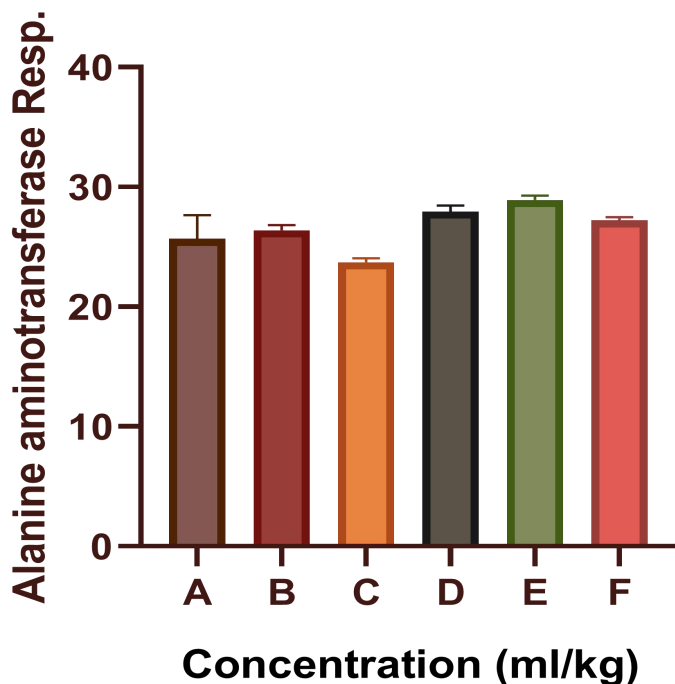


**Fig. 6: Effect of Extract on Glutathione Peroxidase Response**

There is no significant elevation in the level of Glutathione peroxidase within the treatment administered with *Cocos nucifera* at 0.25ml, 0.5ml and 1.0ml respectively on Wistar rat

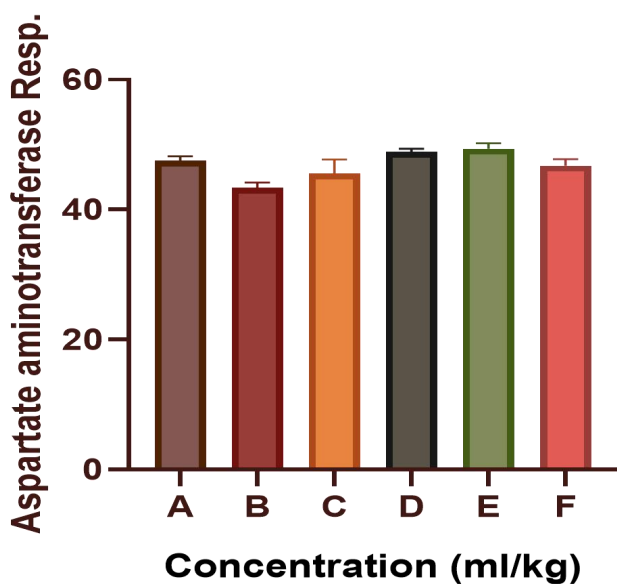
## 4.2 LIVER FUNCTION TESTS

Liver function tests ALT, AST, ALP, GGT, Total bilirubin, Conjugated Bilirubin, Unconjugated Bilirubin and Total protein activity. The eight liver function tests were found to be within normal range in all treated groups.



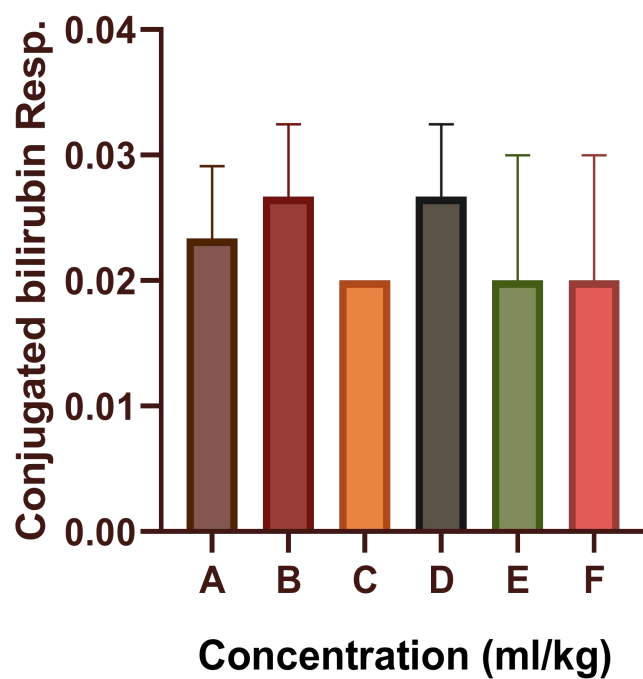
**Fig. 7: Effect of extract on Alanine aminotransferase response in rat challenged with SRBC**

There was an increase in animals treated with the herbal extract than normal animals' control. At 0.25ml dose could enhance ALT response in rat induced SRBC

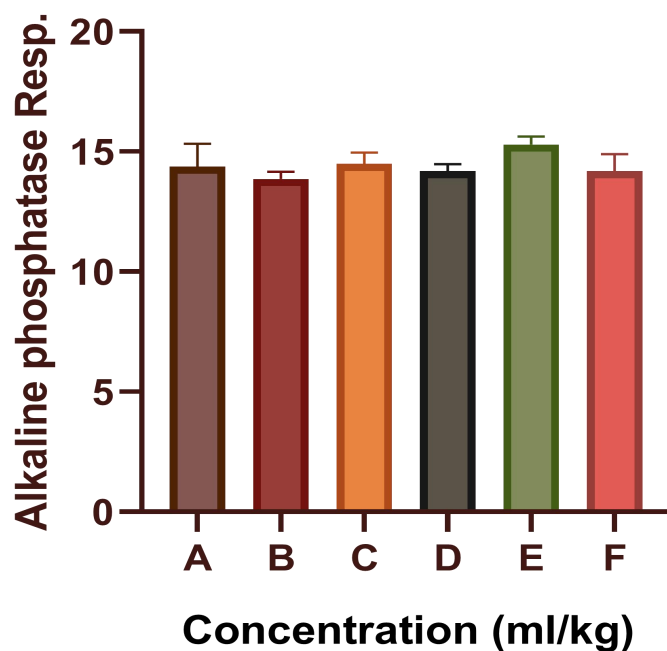


**Fig. 8: Effect of Extract on Aspartate aminotransferase response in Wistar rat induced SRBC**

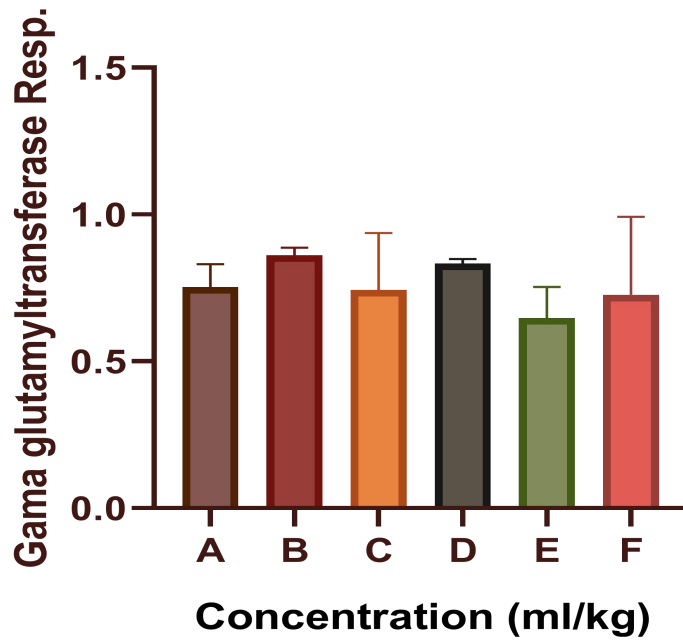
There was no significant response among group D, group E, group F when compared to the normal control group A. There was an increase above the SRBC non-treated group when compared to other groups



**Fig. 9: Effect of Extract on Conjugated Bilirubin Response** in SRBC induced Wistar rat after scarification. Group D administered with 0.25ml extract, when compared with the control could increase the conjugated bilirubin level; compared higher dose of extract reduced the level of conjugated bilirubin when compared with the control group.

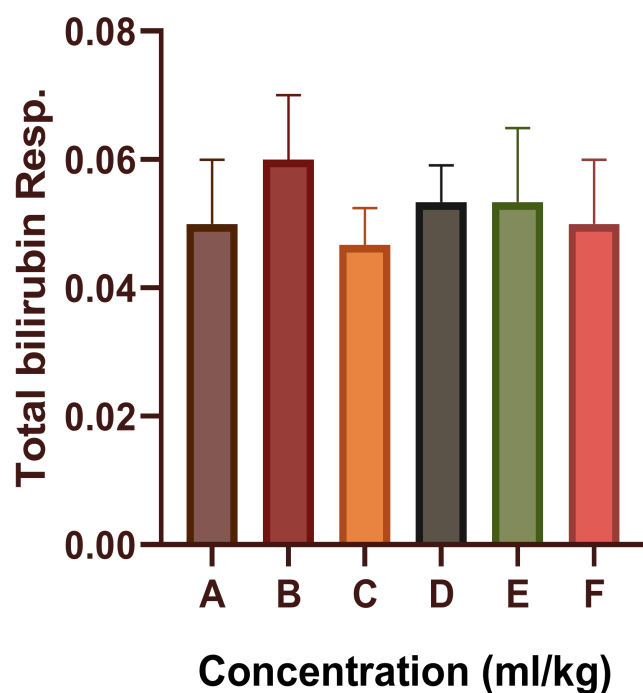


**Fig. 10: Effect of Extract on Alkaline phosphatase level in SRBC induced Wistar rat after scarification.** Group E administered with 0.5ml extract, when compared with the control group, there was a slight increase in the Alkaline phosphatase level; compared with other doses of extract reduced the level of Alkaline phosphatase when compared with the control group.

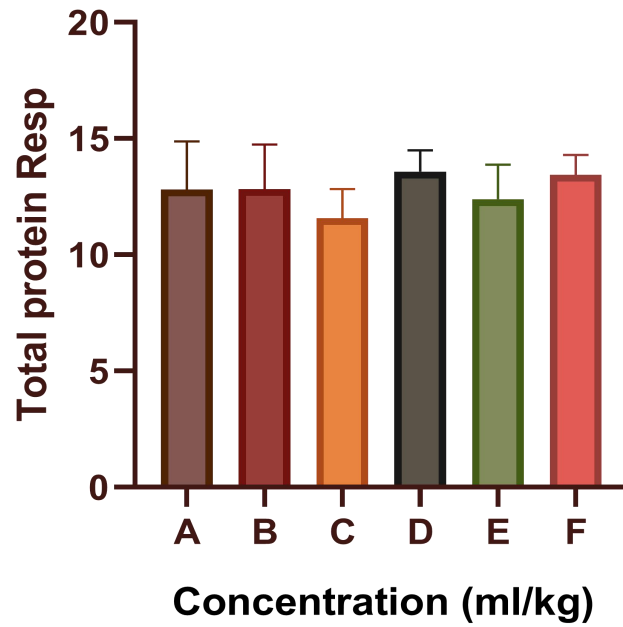


**Fig. 11: Effect of Extract on Gama glutamyltransferase level in SRBC induced Wistarrat after scarification.**

Group D administered with 0.25ml extract, when compared with the control group, there was a slight increase in the Gama glutamyltransferase level; compared with other doses of extract reduced the level of Gama glutamyltransferase when compared with the control group.

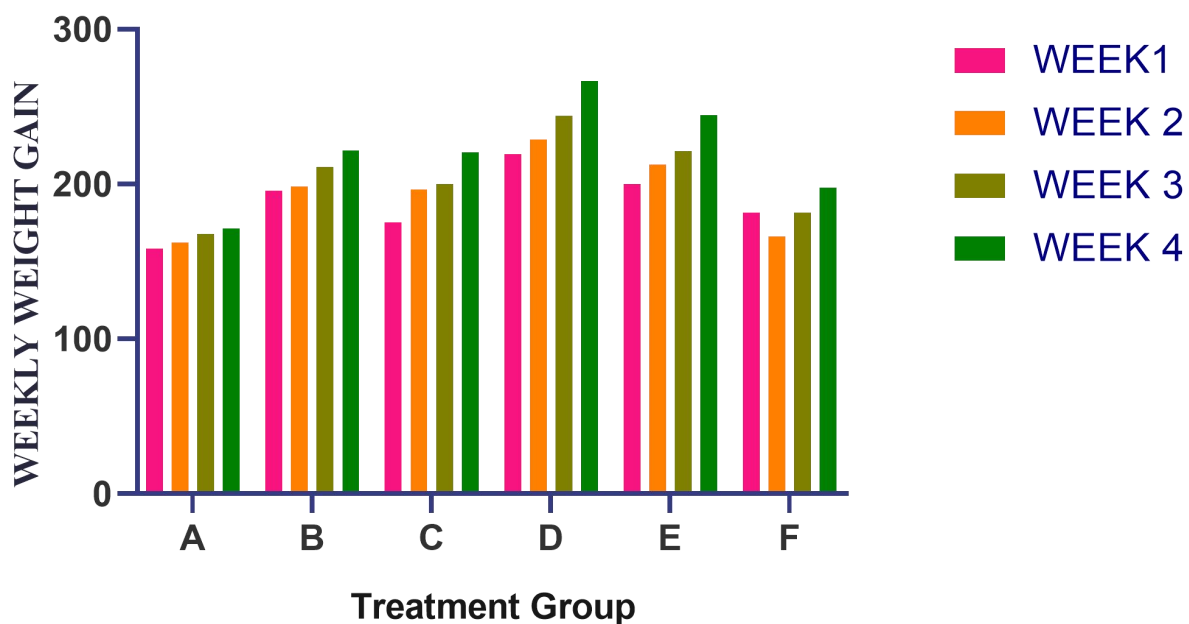


**Fig. 12: Effect of Extract on Total bilirubin level** in SRBC induced Wistar rat after scarification. Group D and E were administered with 0.25ml and 0.5ml extract respectively, when compared with the control group, there was a slight increase in the Total bilirubin level; group B administered with intra-peritoneal in dose 0.3ml SRBC increased the level of Total bilirubin when compared with the control group.



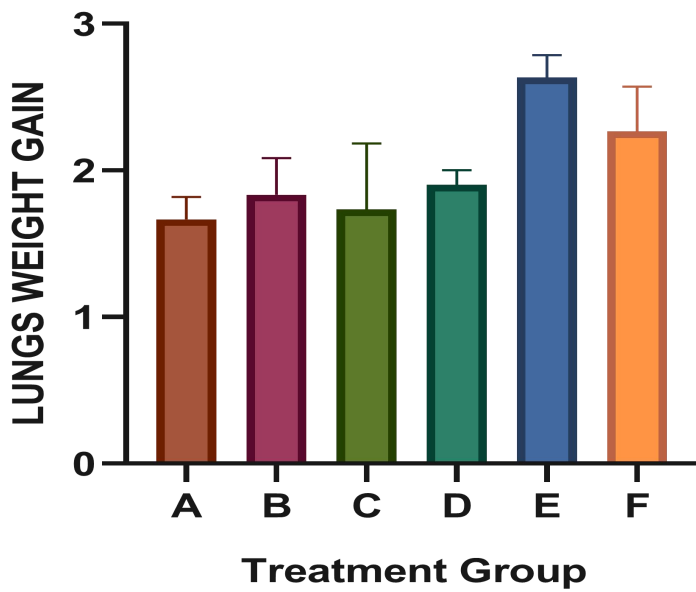
**Fig. 13: Effect of coconut oil on Total Protein Response**

Dose responses on extract in various concentration, group D and F the activity of Total protein level was detected by a slight increase and a decreased in group E when compared with the control group.



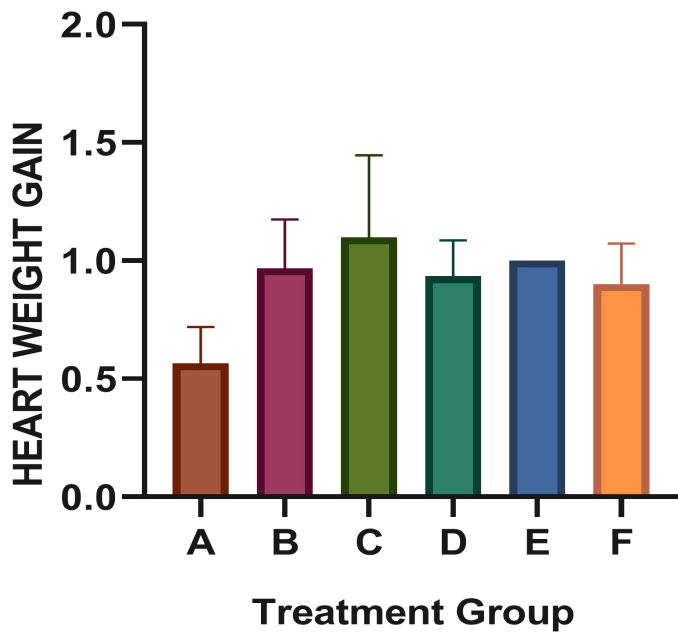
**Fig. 14: The effect weight gain of oil extract from *Cocos nucifera***

Group D, E, F were treated with 0.25, 0.5, and 1ml respectively. Group D having a progressive significant increase in weight gain when compared with the control group A. Group F having the highest dose of oil extract. Resulted in weight loss during the second week of administration



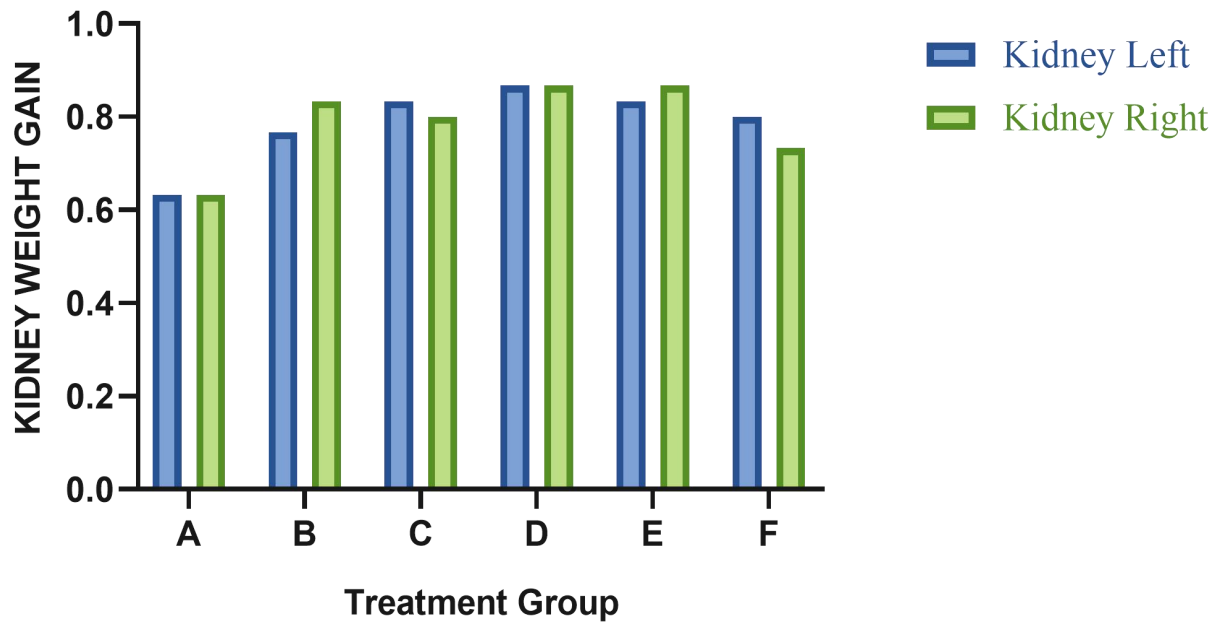
**Fig. 15: The effect of oil extract from *Cocos nucifera* plant on lung weight gain**

There was a significant increase in Lungs weight in group E when compare with the control group



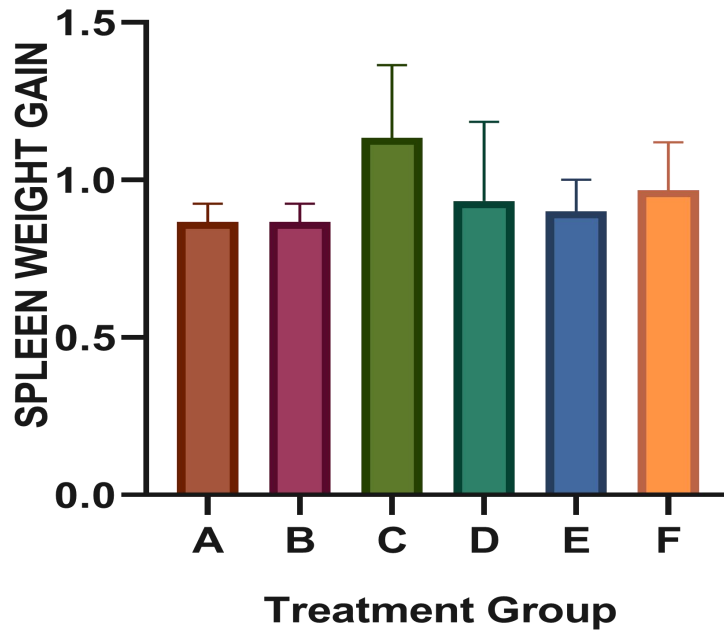
**Fig. 16: The effect of the extract on heart weight gain**

There was an increase in group E when compared to the other doses of the extract. Group C has the highest heart weight gain when compared to control.



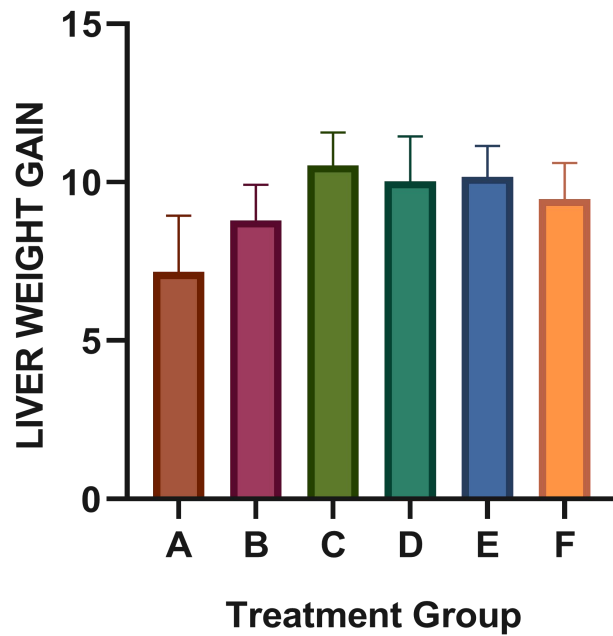
**Fig. 17: The effect of the extract on both kidneys**

There was a progressive increase among the treatment group when compared to the control group



**Fig. 18: Effect of *Cocos nucifera* oil extract on Spleen weight gain**

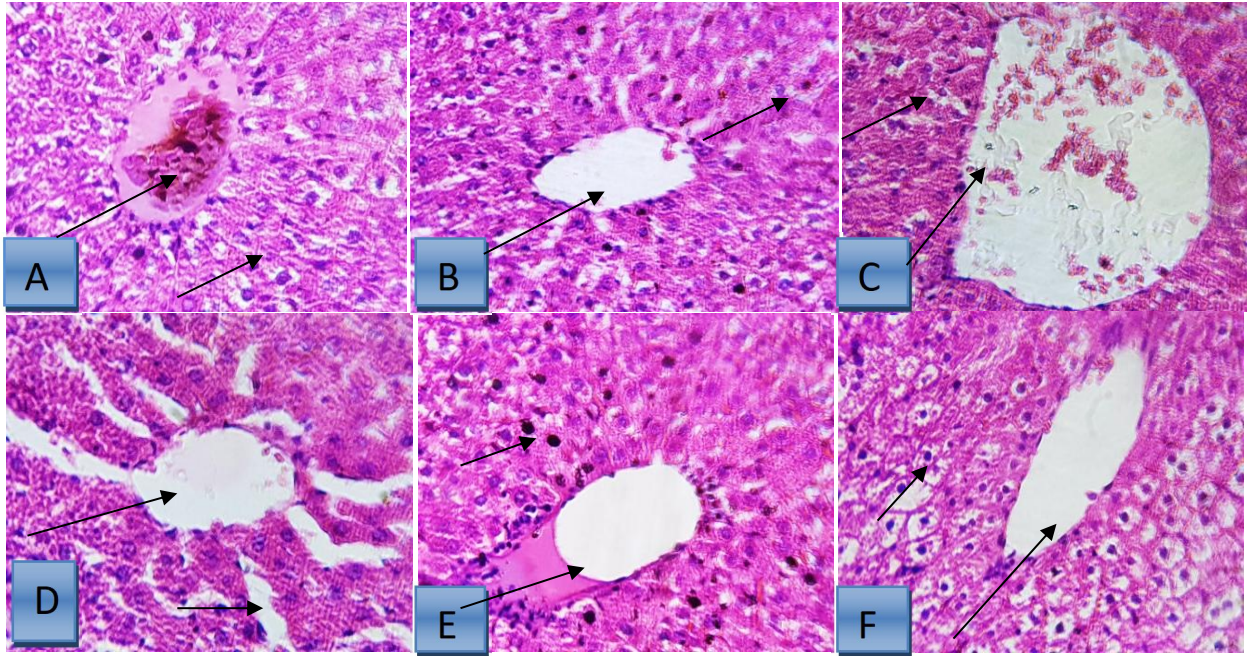
The above figures show various spleen weight gain got by the animal during the experimental period of 21 days of administration. Group A serves as the normal control, group B serves as induced SRBC negative control, group C serves as a standard drug Azathioprine, while group D, group E and group F received 0.25ml, 0.5ml and 1.0ml respectively. There were significant different



**Fig. 19: Effects of various doses of *Cocos nucifera* oil extract response in Wistar rat**

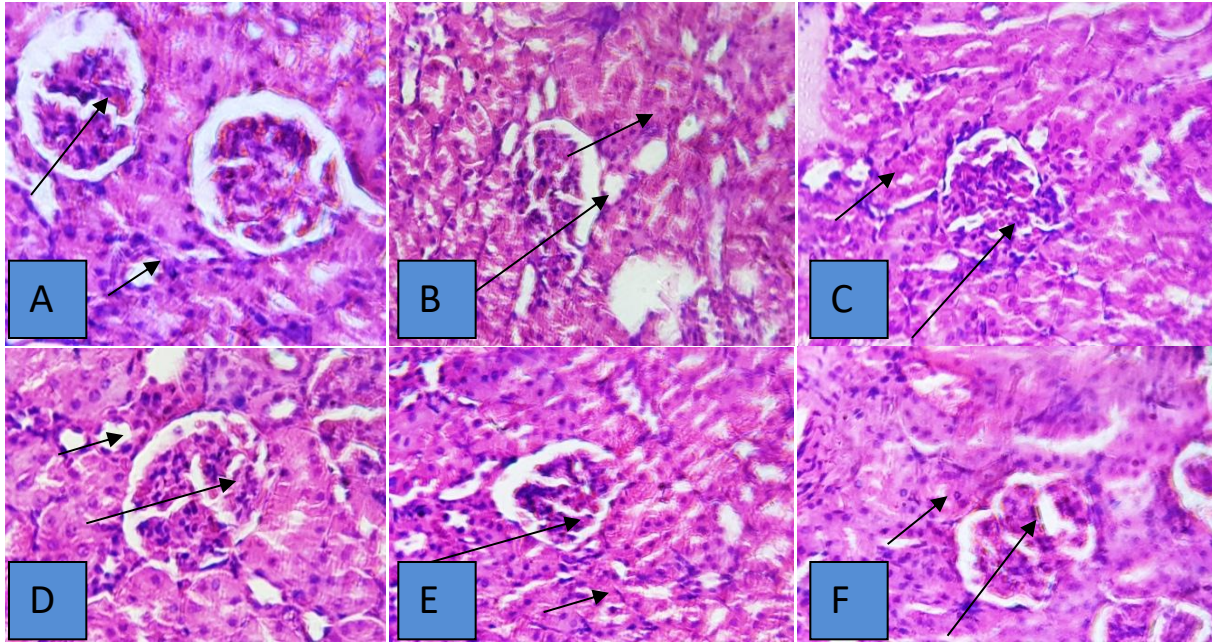
Azathronpine group C having  $10.53 \pm 0.601$  compared with control group having  $7.167 \pm 1.027$   $P < 0.05$  considered being a significant difference when compared with the control animals

### 4.3 HISTOLOGICAL ANALYSIS



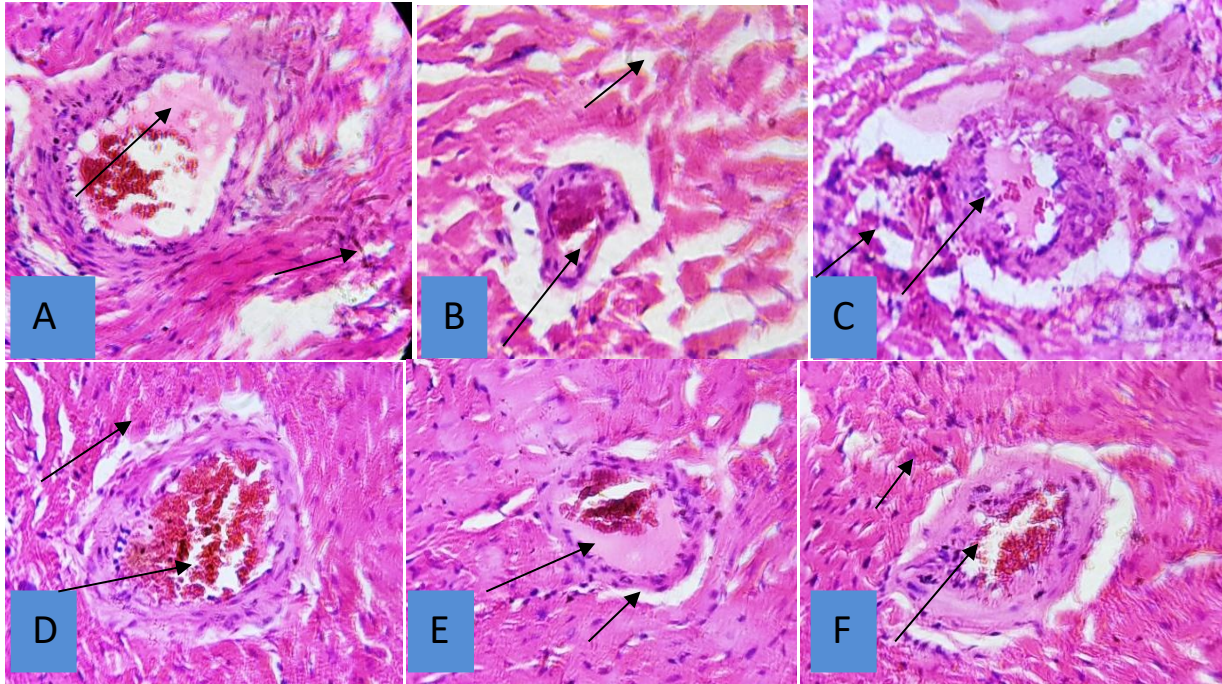
**Plate 2:** Effect of *Cocos nucifera* oil extract on histology of the Liver

- A. LIVER: reveals visible centriole (long arrow) with the hepatocytes revealing pyknotic nucleus (short arrow).
- B. LIVER: reveals centriole surrounded by mild inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).
- C. LIVER: reveals centriole (long arrow) surrounded by hepatocytes that appear not so distinct. (Short arrow).
- D. LIVER: reveals distinct centriole (long arrow) with the hepatocytes and dilated sinusoidal (short arrow).
- E. LIVER: reveals centriole surrounded by focal inflammatory cells (long arrow) with the hepatocytes revealing mild steatosis (short arrow).
- F. LIVER: reveals centriole (long arrow) surrounded by hepatocytes with hydropic fatty changes and revealing mild steatosis (short arrow).



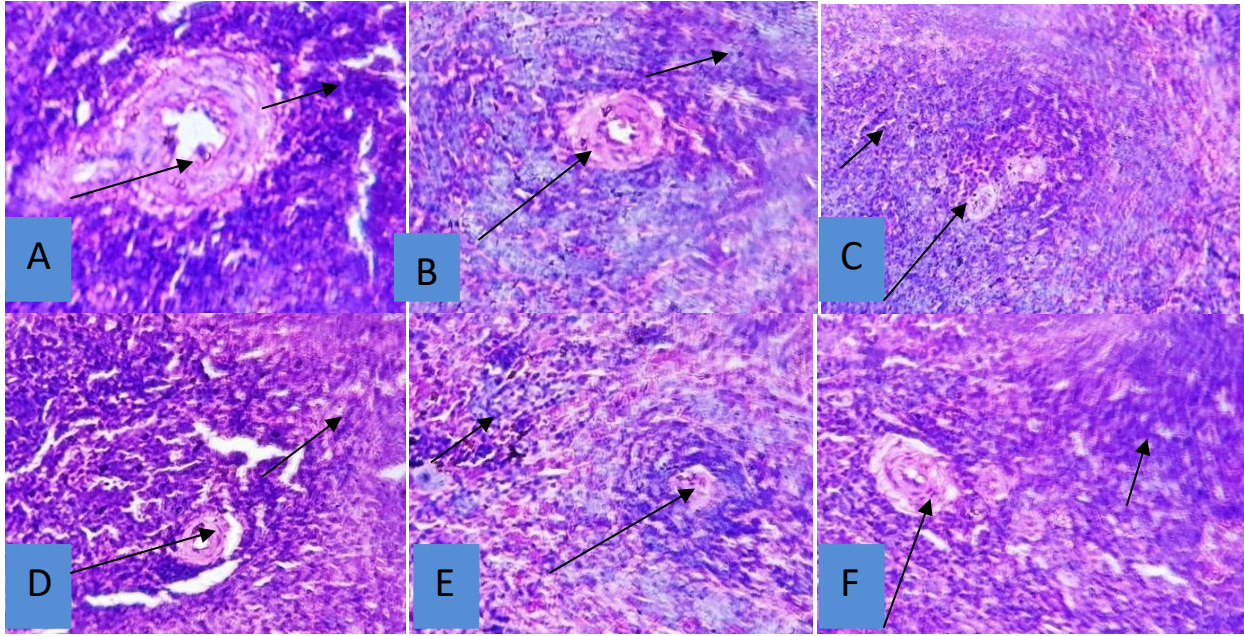
**Plate 3:** Effect of *Cocos nucifera* oil extract on histology of the Kidney

- A. KIDNEY: reveals prominent renal corpuscle (long arrow) and interstitial space and tubules (short arrow).
- B. KIDNEY: reveals atrophied renal corpuscle (long arrow) and interstitial space with mononuclear cells (short arrow) and mild tubular necrosis.
- C. KIDNEY: reveals renal corpuscle appearing not so distinct with granulated nucleus (long arrow) and interstitial (short arrow) and tubular necrosis.
- D. KIDNEY: reveals visible renal corpuscle (long arrow) and interstitial space (short arrow) and tubules.
- E. KIDNEY: reveals atrophied renal corpuscle (long arrow) and interstitial space with mononuclear cells (short arrow) and tubular necrosis.
- F. KIDNEY: reveals renal corpuscle appearing not so distinct (long arrow) and interstitial (short arrow) and tubular necrosis.



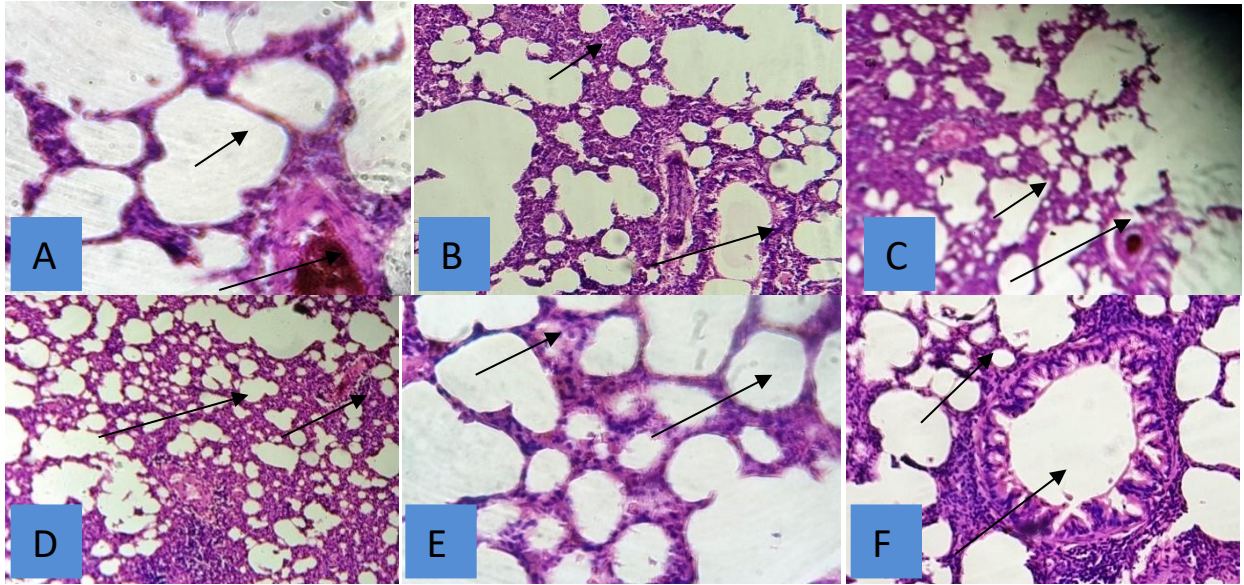
**Plate 4:** Effect of *Cocos nucifera* oil extract on histology of the Heart

- A. Heart composed of bundles of myocardial fibres (short arrow), interstitial space and prominent coronary artery (long arrow)
- B. Heart composed of bundles of myocardial fibres (short arrow), interstitial space and atrophied coronary artery (long arrow)
- C. Heart composed of bundles of myocardial fibers (short arrow), interstitial space and congested coronary artery (long arrow)
- D. Heart composed of bundles of myocardial fibers (short arrow), interstitial space and large prominent coronary artery (long arrow)
- E. Heart composed of tight bundles of myocardial fibers (short arrow), interstitial space and coronary artery (long arrow)
- F. Heart composed of bundles of myocardial fibers (short arrow), interstitial space and coronary artery (long arrow)



**Plate 5:** Effect of *Cocos nucifera* oil extract on histology of the Spleen

- A. The spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear distinct.
- B. The spleen shows lymphoid follicles (short arrow) with centrally to eccentrically located large blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which. The red pulps appear coarse.
- C. The spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps appear coarse.
- D. The spleen shows prominent lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps are prominent.
- E. The spleen shows lymphoid follicles (short arrow) with not so prominent eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes. The red pulps are appeared coarse.
- F. The spleen shows lymphoid follicles (short arrow) with eccentrically located blood vessels (long arrow). The follicles (white pulp) comprise aggregates of lymphocytes which appear activated. The red pulps are appeared coarse.



**Plate 6:** Effect of *Cocos nucifera* oil extract on histology of the Lung

- A. Lung histology with prominent blood vessel (long arrow) and visible alveolar ring. The alveolus appears distinct (short arrow)
- B. Lung histology with prominent bronchiole (long arrow) and visible alveolar ring. The alveolus appear slightly congested (short arrow)
- C. Lung histology with prominent blood vessels (long arrow) and visible alveolar ring. The alveolus appear slightly congested (short arrow)
- D. Lung histology with prominent alveolar sac (long arrow) and alveolar ring. The alveolus appear slightly congested
- E. Lung histology with prominent alveolar sac (long arrow) and alveolar ring. The alveolus appear slightly congested
- F. Lung histology with prominent bronchiole (long arrow) and visible alveolar ring. The alveolus appear slightly congested (short arrow)

## CHAPTER FIVE

### DISCUSSION

The immune response is a biological process which depends on external fortification and is never same for all members of an immunized population. The immune reaction is influenced by many genetic and ecological factors; the series of immune responses in a large random population of faunas follows a standard distribution. However thousands of plants have the ability to treat and cure illnesses, but only few plants have been discovered scientifically to explain its therapeutic properties.

Hematological parameters were carried out for, Red Blood Cell, White Blood Cell, Lymphocyte, haemoglobin, Platelet and other parameters. Geo and Baskaran 2008 study reveal that coconut protein was able to increase the level of WBC, RBC and Platelet count which signify its immunomodulatory activity. The white blood cell (WBC) counts showed in table 1, the value of group D  $11.70 \pm 1.25$  increase when compare group C and F extract administration, the value dropped for group E  $9.90 \pm 0.76$  control group having  $11.73 \pm 1.51$ . Immunosuppressant comprises an act that decreases the activation or efficiency of the immune system. Bone marrow is the supple tissue found in the hollow inner of bones. In older persons, marrow in big bones yields new blood cells. When it is in a suppressed condition WBC count level gets reduced. However, group D, when compared with the control group, resulted in a slight increase  $9.90 \pm 1.34$  and group E resulted in  $8.20 \pm 0.50$  decreased in the lymphocyte count when compared to the  $9.77 \pm 1.39$  control group. There is a significant increase in haemoglobin level group D  $15.57 \pm 0.67$  when compared with the control group A  $13.60 \pm 0.53$ . The RBC value, no significant differences occurred among the various group. Lymphocyte, additional vital cells of the immune

system are responsible to actuate production of granulocyte (type of a WBC) to destroy the antigen (Prescott, 1999). Increment level in lymphocyte has been found in experimental groups A, B and C having  $9.77 \pm 1.39$ ,  $8.53 \pm 0.18$  and  $9.90 \pm 1.34$  respectively. The value of granulocyte increases in group D  $8.0 \pm 0.67$  against group A  $7.40 \pm 1.70$ . There is a significant increase in group D when matched with group A in hemoglobin and hematocrit level resulting in  $13.60 \pm 0.53$ ,  $15.57 \pm 0.67$  and  $37.50 \pm 1.47$ ,  $42.37 \pm 1.48$  respectively. At a higher dose of oil extract, there was a substantial increase in various platelet counts, group F showing  $996.30 \pm 225.90$  when compared with the control group A  $740.00 \pm 72.76$ .

Human immunodeficiency virus (HIV) contagion is characterized by a reduction and eventually, exhaustion of CD4<sup>+</sup> T-lymphocytes (assistant T cells). By using immunophenotyping, patient samples are tested for the percentage of lymphocytes that are B cells, natural killer (NK) cells, T cells, CD4<sup>+</sup> T cells (assistant T cells), and CD8<sup>+</sup> T cells (suppressor T cells). This is achieved by incubating anti-coagulated whole blood with monoclonal antibodies to the several cellular antigens that recognize specific cell populations (phenotypes), and then breakdown the blood into smaller molecules remove red blood cells (Ford *et al.*, 2017). Though, CD4 and CD8 cell count provides preeminent direct measurement of a patient's immune position and danger for opportunistic infections, and it remains a vital test regarding analytic decision-making, mainly for patients with progressive HIV disease. In this study, results showed that high and low dose of extract-treated animals increase the CD4 Count level significantly when compared to control. Group D,  $7.763 \pm 0.185$ , when compared with the negative control brought down the immune system group B  $5.643 \pm 0.558$ , with the control group A having  $7.830 \pm 0.894$ . The SRBC reduced the antibody response significantly when compared to the control group, a lower dose

could boost the immune system. The extract trigger the CD4 and CD8 cells count which effect the T-cell process, resulting in an increase of T-cell immune reaction significantly.

The level of Malondialdehyde (MDA) in the liver homogenate was studied (Uchiyama *et al.*, 1978). MDA level was considerably increased in group F  $41.08 \pm 0.629$  when compared to the control  $36.14 \pm 3.681$ . At a lower dose of extract, it brought down the level of MDA to  $34.53 \pm 1.160$ . The higher dose of extract could boost the MDA level (Table 1). It has been reported that treatment with coconut oil in rats increased the activities of antioxidant enzymes (Iranloye *et al.*, 2013). In the antioxidant enzymes systems, CAT activity was significantly increased in group D  $241.0 \pm 12.61$  when compared with group A having  $228.6 \pm 11.62$ . The activity of CAT significantly increased in group B  $252.5 \pm 21.17$  when compared to the control ( $p < 0.05$ ), however, CAT levels were significantly reduce in group F ( $222.2 \pm 7.25$ ) was observed when compared with the control. The activity of SOD, there was no significant increase among treated animals when compared to the control. Evaluation carried out by (Yeap *et al.*, 2015) reveal that, coconut oil decrease lipid peroxidation and increases the activity of SOD in the blood serum of mice. The SOD value in this study reveals that group D having  $1.881 \pm 0.061$  increase when compared with the control  $1.417 \pm 0.161$  while group F had no influence on the activities of SOD when compared with the control. Lastly, Glutathione peroxidase activity, there was a significantly reduced in group C  $129.0 \pm 1.127$  when compared with the control of  $137 \pm 5.875$ . Studies on the natural effects of coconut oil have verified that it improves oxidative pressure by enhancing the antioxidant defence system, cleaning up free radicals and decreasing lipid peroxidation (Nevin *et al.*, 2006 and Van *et al.*, 2004). Antioxidant enzymes are extremely important part of cellular defense against reactive oxygen species (ROS) and finally oxidative

stress. The Antioxidants enzymes involved in the removal of ROS include GSH, CAT, and SOD respectively.

An excess of these enzymes such as, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are raised up in serum when liver cells have been injured. The effect of extract of *Cocos nucifera* on liver function parameters such as ALT, AST, ALP, GGT, TP, UB, Cong. Bil and Tot. Bil (Figures 7, 8, 9, 10, 11, 12, and 13). One of the significant finding in (Djami *et al.*, 2011) studies was an increase in liver enzymes that plays a role in protein metabolism. Administration of extract group E increased the values of ALT, AST and ALP resulting in  $28.91 \pm 0.215$ ,  $49.35 \pm 0.510$  and  $15.28 \pm 0.199$  when compared with the control group  $25.67 \pm 1.145$ ,  $47.51 \pm 0.403$ , and  $14.37 \pm 0.552$  respectively, which resulted in a decrease in group F administered high extract dose. There was no significant difference at ( $P < 0.05$ ) on conjugated bilirubin value when compared with the control. Group D shows an increase in the GGT, TOT BIL AND TP antibody level  $0.833 \pm 0.009$ ,  $0.053 \pm 0.003$  and  $13.57 \pm 0.529$  when compared with the control  $0.753 \pm 0.045$ ,  $0.050 \pm 0.006$  and  $12.80 \pm 1.201$  respectively.

The bodyweight of the animals of a control group and that of treatment groups are given in fig 14, the graph shows the weekly weight gain of the rat during the period of administration to the day of sacrifice. At a lower concentration (0.25ml) of oil extract, the animals reveal an increased body weight gain, with a decrease in higher concentration (1.0ml). Similarly, important organs like liver, 0.25ml dosage showed an increased mass of liver when compared to higher dose of the oil extract and a control group. Whereas at a higher dose (1.0ml) concentration resulted in an increase in spleen organ weight, there was a significant increase in 0.5ml azathioprine in spleen and heart weight gain. Also, 0.5ml oil extract resulted in a significant increase in lung weight gain.

The histology of the various organs such as the kidney, liver, spleen, lungs and heart as shown in plate 1, 2, 3, 4 and 5 respectively. Jeffery *et al.* (1997) demonstrated that spleen lymphocytes propagation was enhanced by palmitic acid rich food of saturated fatty acid found palm oil. Histology of the liver of the control group A was used to compare the various doses of oil extract and the effect of the SRBC in the rat. Group D, E and F were administered with 0.25ml, 0.5ml and 1.0ml coconut oil extract respectively. The presence of the antigen in the bloodstream reveals inflammatory cells with mild steatosis in group B induced SRBC, none treated with oil extract. Group D reveals distinct centriole and dilated sinusoidal, no inflammatory cells, no steatosis, Group E resulted in focal inflammatory cells and mild steatosis. Group F reveals hydropic fatty changes and mild steatosis in the nucleus as compared with the control group visible centriole with hepatocytes revealing pyknotic nucleus which shows a delay in making the blood smear after blood collection. At a lower dose of oil extract, there was a significant enhancement in the liver cell. Kidney cells reveal necrosis in group B, C, E and F while group D has no necrosis when compared with the control having no necrosis, there is a significant difference in group D been able to boost the immunity of cell in the kidney to fight against. The differences between the heart occurred in the coronary artery which state that group A has a prominent coronary artery, group B atrophied coronary artery, group C congested coronary artery, group D large prominent coronary artery, group E and F coronary artery. At 0.5ml oil extract, tight bundles of myocardial fibers occurred. The red pulps present in the spleen's organ appear coarse in group A, while group B, C, E and F the red pulp appear to be coarse in group D red pulp are prominent. The alveolus present in the lungs appear to be distinct in group A and congested in group B, C, D, E and F.

## 5.1 CONCLUSION

This study has shown that the immune reaction is enhanced upon by oral administration of *Cocos nucifera* oil extract. It is clear from the results that lower dosage of oil extract mainly 0.25ml has the ability to activate both cellular and humoral immune reaction. However, 1.0ml of oil extracts which was the highest dose has resulted in a negative response to enhance the immune response. In Ayurvedic practice, dose-effect is achieved as per the requirement; the discipline of herbal drug can cause a change in action based on the dose of the drug administered. Summarily, the oil extract tested has obviously revealed its immunomodulatory result when used at a lower dosage. Finally, *Cocos nucifera* oil could boost the immune structure at lower concentration and it should also be kept in mind that higher dosage of same extract could result in a physiological effect in the human body.

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