THE EFFECT OF OCIMUM TENUIFLORUM (NCHUANWU) LEAF EXTRACT ON HEMATOLGICAL PARAMETERS OF IMMUMNOSUPPRESSED ALBINO RATS.

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DEDICATION

This research work is dedicated to God Almighty who saw me through the whole process.

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I give thanks to God almighty who started this good work and have completed it, to God be the glory.

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ABSTRACT

The effect of *Ocimum tenuiflorum* on hematological parameters in immunosuppressed albino rats was investigated in this study. The aqueous (AE) and methanol extract (ME) of the leaf were obtained. Acute toxicity study was done to determine the LD_{50} of the leaf extract. Rats of mixed sexes, aged 2-3months, weighing 150 to 240 grams were used. The rats were divided into 8 groups A-H of four rats each. Group A served as normal control. Immune-suppression was induced using 3mg/kg bodyweight of cyclophosphamide (CP) to rats in groups B to F for 5 days. Group B was CP control. Group C, D, E and F received 500 mg/kg bodyweight (b.wt) of AE, 1000mg/kg b.wt of AE, 500 mg/kg b.wt of ME, and 1000 mg/kg b.wt of ME once daily for 21 days by oral route, using oral cannula. Groups G & H (none induced) received 500 mg/kg b.wt AE and 1000 mg/kg b.wt ME once daily for 21 days. Rats in all the groups received rat feed and water *ad libitum* for 21 days. On days 15 and 22, 2ml of blood were drawn from each rat via the median canthus into EDTA (ethylenediamin tetra acetic acid) bottle. The HB, PCV, total and differential WBC, were determined using automatic cell counter. Group B CP control of day 15 showed significantly deceased value in HB; 5.8 ± 0.30g/dl, PCV; 17.6 ± 0.74%, WBC; 0.63 ± 0.2x$10^{3/\mu l}$ Neut; 0.75 ± 1.50%, Lym; 3.5 ± 4.73%, compared with the normal control group A: HB; 13.65 ± 0.47g/dl, PCV;41.15 ± 1.35%, WBC; 11.35 ± 1.76 x$10^{3/\mu l}$, Neut; 22.25 ± 6.55%, Lym; 73.75 ± 6.75, Mono; 2.5 ± 1.73, Eos; 1.75 ± 1.26, ($P<0.001$). Groups C, D, E and F, of day 15 had significant increase in (Hb); 11.27 ± 0.85g/dl, 12 ± 0.30g/dl, 11.67 ± 0.38g/dl, 12.45 ± 0.31g/dl when compared with CP control group B: HB value; 5.88 ± 0.30g/dl ($P<0.05$). Groups C, D, E and F had significantly increased PCV; 33.47 ± 2.17%, 36.53 ± 0.50%, 35,5 ± 1.67%, 37.35 ± 1.12%, when compared with CP control group B; PCV value; 17.58 ± 0.74%, ($P<0.01$). (WBC) count on day 15 were increased in groups C, D, E and F, values were; 23.87 ± 24.16 x $10^{3\mu/l}$, 12.07± 2.8 x $10^{3\mu/l}$, 14.97 ± 4.24 x $10^{3\mu/l}$, and 14.3 ± 7.40 x $10^{3\mu/l}$ compared with the CP control B; 0.63 ± 0.21 x $10^{3\mu/l}$,($P<0.001$). Lym count was significantly raised in groups C; 30 ± 14.73%, D; 42 ± 14.18%, E; 54.67 ± 8.08%, F; 21.75 ± 11.21%, when compared with the CP control group B; 3.5 ± 4.73%, ($P<0.001$) Neutrophil count revealed very high count in groups C; 63.33 ± 10.60%, D; 54.33 ± 15.70%, E; 38.33±6.66%, F; 77.75±11.00%, when compared with the CP control 0.75±1.50% ($P<0.001$). On day 22, there was an increase in

neutrophil count in groups D; 60±14.40%, and F; 52%, when compared with group B; 38±4.24% ($p<0.001$). Lymphocytes count were significantly increased in groups C; 55.5±12.02%, and E; 60±7.07%, when compared with the CP control group B; 54.64±7.57%, ($P<0.05$). Monocyte count was significantly raised in groups C; 2±0.00%, D; 1.15±2.12%, and F; 2%, when compared with control group B; 1±1.00%, ($P<0.05$) Eosinophil count, were significantly high in groups C; 5±7.07% and E; 4±1.41%, when compared with the CP control group B; 1.33±1.53%, ($P<0.001$). The HB concentration revealed significant increase in groups C; 11.65±0.21g/dl, D; 13.15±0.49g/dl, E; 12.25±9.07g/dl F; 13.43±1.33g/dl when compared with the CP control group B; 5.633±0.85g/dl, ($P<0.05$). The pack cell volume showed significantly increased value in groups C; 35.2±0.85%, D; 39.85±1.34%, E; 36.95±0.21%, F; 41.9%,when compared with the CP control B; 17.43±2.23%, ($P<0.01$). The phytochemical analysis of the aqueous and methanol extract of *Ocimum tenuiflorum* reveal the presence of flavonoids, terpenoids, alkaloids, carbohydrates, proteins, reducing sugar, glycosides, saponin, tannin, protein, steroids, oil, resin, and acidic compound. The histological analysis of the liver and spleen of the normal control showed normal features of the hepatic and splenic tissues. The treated group and none induced that received 500mg/kg AE and 1000mg/kg ME showed normal features of the two organs, with mild histopathological changes, which could still be the effect of CP. *Ocimum tenuiflorum* leaf of aqueous and methanol extract has mild effect on the liver and spleen of albino rats at dose used in this study; hence it is safe for use in traditional medicine. In conclusion this study has revealed that *Ocimum t.* extract has the ability to boost suppressed immunity.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Immunosuppression is the suppression of the immune system and its ability to fight infections. It involves an act that reduces the efficacy of the immune response (Sigal and Dumont, 1992). Immunosuppression may result from certain diseases such as AIDS or lymphoma, lupus or from certain drugs, used to treat cancer (for example cyclophosphamide). Immunosuppression may occur as an adverse reaction to treatment of auto-immune diseases like rheumatoid arthritis or Crohnôs disease (Abbas, *et al*, 2010). It may be deliberately induced with drugs, to prevent the body from rejecting an organ or treating graft ï versus host disease after bone marrow transplant, drugs such as steroids, azathioprine, ciclosporin monoclonal antibodies which include basiliximab, zenapax, muromonab, and prednisone are used in organ transplant (kidney, liver, heart) these drugs are also known as immunosuppressant drugs, they inactivates immune system to prevent tissue rejection (Halloran, 2004). Immunosupprression may occur as a result of radiotherapy. Non-deliberate immunosuppression can occur in malnutrition, aging, many types of cancer such as leukemia, lymphoma, multiple myeloma and certain chronic infections such as Human Immunodeficiency Virus (HIV) which results in increased susceptibility to pathogens such as bacteria and virus (Abbas *et al* 2010). Immunosuppression was deliberately induced in this research to evaluate the effect of *Ocimum tenuiflorum* leaf extract on immune system. **The immune system:** Immune system protect the body by recognizing and responding to antigens like bacteria, fungi, viruses, chemicals, toxins, by attacking and destroying them in the body. To function effectively an immune system must detect a wide variety of

pathogens and distinguish them from the organismôs healthy tissues (Beck, *et al*, 1996).

Cyclophosphamide was used in the course of this study to induce immunosuppression in albino Wister rats. Cyclophosphamide is an alkylating drug used primarily for the treatment of several types of cancer. The active compounds acrolein and posphamide slows down the growth of cancer cells by interfering with the action of deoxyribonucleic acid (DNA) of the cancerous cell. It suppresses B-cell, that activates antibody formation. While performing its functions, some healthy cells were destroyed as well, causing serious side effect that lead to immune suppression (Starzl *et al*, 1993).

In this research, the effect of *Ocimum tenuiflorum* on immunosuppression was investigated. *Ocimmum tenuiflorum* leaf is a latin word meaning ñbasil with small flower.ôô It is also called *Ocimum sanctum* (ôôsacred frangrant lipped basilôô) or *Ocimum gratissimum* (ôôvery grateful basilôô) (Steven, 2004). If the source is from India it is called *tulsi.* While Igbos in the Eastern Nigeria call it ñ*Nchuanwu, Ahigbe,* or *Alili*ò. *Ocimum tnuiflorum* is a perennial herbs, it is an erect much branched sub shrub, 30-60cm tall with hairy stem. It is basically among the 50 to 150 species of the genus *Ocimum (*Warrier, 1995). *Ocimum tenuiflorum* belong to the family of lamieceae (Staple *et al*, 1999), an herb and a shrub found at the tropical region of Asia. The herb is now grown worldwide including Nigeria. *Ocimum tenuiflorum* is widely cultivated in India for religious and medicinal purposes and for its essential oil (Socorro *et al* 2002). It bears green, dark green and purple leaves. The green leaf is the most common (Steven, 2004)*, Ocimum tenuiflorum* is strongly scented (Kothari *et al,* 2005). The leaves are mostly used as spices for stew, yam porridge and for frying of meat.

In India there is a form of healing called Ayuveda, *Ocimum tenuiflorum* is a well known sacred plant used for such healing. It is also called holy basil (Warrier 1995). Ayur means óôlifeôô and veda means óôknowledgeôô. Therefore Ayurveda means knowledge of life (Steven 2004), including health and disease, (Puri *et al*, 2002). In Asian country no good herbalist can prepare herbs without adding *Ocimum tenuiflorum.* It is so sacred to Asians that some part of Asian countries worship *Ocimum,* (Chatterjee and Gautam , 2001). The nutritional components of *Ocimum tenuiflorum* are; vitamin A and C, minerals; calcium, iron and zinc, as well as chlorophyll (Anbarasu and Vijayalakshmi, 2007). The leading phytochemical compounds are: Alkaloid, glycoside, saponin, Tannins, Flavonoid, resin, Protein, Steroid, Reducing sugar, Oil, and Terpenoid, carbohydrate and acidic compound. Other active compounds include: Eugenol (volatile oil),Ursolic acid (triterphenoid), Rosemerinic acid (phenylpropanoid). caryophylene and oleonolic acid, linolenic acid, and linoleic acid, (Shishodia *et al* 2003). *Ocimum tenuiflorum* was believed to have lots of health benefits. People take holy basil to increase good health, prevent and treat diseases (Prakash*, et al,* 2005). Its uses and benefits are related to the various human body systems. Based on observations made by practitioner and modern scientific research, it shows that *Ocimum tenuiflorum* act on the following body system; cardiovascular and circulatory system (heart, blood circulation) (Sood, *et al,* 2006). Digestive system: (oesophagus, stomach, intestines, liver, and pancreas). Immune system: (inflammatory response, white blood cells, lymph glands) (Mukhehjee *et al,* 2005). Skin system: (skin, sweat) (Shetty *et al,* 2006). Muscular system. Nervous system: (brain, spinal cord, nerve). Respiratory system: (nose, trachea, lungs). Reproductive system (Ahmed, *et al*, 2002) and urinary or excretory system. In ayurveda remedies tulsi extracts were used to treat common cold, headache,

diarhoea, stomach disorders, inflammations, heart disease, various form of poisoning, typhoid and malaria.

Traditionally tulsi is taken as an herbal tea. In ancient China tonic herbs were used by the wisest and wealthiest people, to maintain wellness because it makes people appear younger and increases body vitality. It induces appetite, enhances motor activity and physical performance, increases the bodyôs efficiency in using oxygen, and enhances protein syntheses (Steven, 2004). It balances and regulates cortisol level and induces release of adrenal hormones (Puri *et al*, 2002). Modern clinical studies also agree with claims from ayurveda literature.

THE SIGNIFICANT OF THIS STUDY

Due to the importance of immune system in life and lack of awareness about the medicinal values of *Ocimum tenuiflorum* leaf in this part of the country, this research was desighned.

1.2 AIM AND OBJECTIVES

AIM

To determine the effects of *Ocimum tenuiflorum* (Nchuanwu) leaf extract on hematological parameters of immune suppressed albino rats.

OBJECTIVES

- To extract *Ocimum tenuiflorum* leaves using aqueous and methanol as solvents.
- To identify the phytochemical components of the leaf extract of *Ocimum tenuiflorum.*
- To determine the LD 50 of *Ocimum tenuiflorum* in albino rats.
- To administer the extracts orally to the albino rats.
- To assess the haematological parameters of the albino rats

- To compare the data generated statistically.
- To assess the effect of the leaf extract on the histology of the liver and spleen.

OCIMUM TENUIFLORUM

Ocimum Tenuiflorum (Photo Gourange uk)

CHAPTER TWO

LITERATURE REVIEW

2.1 **IMMUNOSUPPRESSION**

Immunosuppresion involves the act that reduces the efficacy of the immune system and its ability to fight infection. Many immunosuppressive measures aimed at slowing the proliferation of activated lymphocytes (Sigal, 1992). In this condition immune system is inactivated or made inefficient to recognize antigens in the body, For example, bacteria, virus, fungi, et cetera. Immunosuppresion may result from certain diseases such as AIDS, lymphoma, leukemia, multiple myeloma, and certain chronic infections like Human Immune Deficiency Virus (HIV) (Abbas, *et al*, 2010). Immunosuppression may also occur as a result of adverse reaction of certain drugs such as some of those used to treat cancer (Sigal and Dumont, 1992). The unwanted effect in non-deliberate immunosuppression is immunodeficiency that results in increased susceptibility to pathogens such as bacteria and virus. Immunodeficiency is also a potential adverse effects of many immunosuppresant drugs (Starzl *et al*, 1993). In this sense, the scope of the term immunosuppression in general includes both beneficial and potential adverse effects of decreasing the function of the immune system, whereas the term immunodeficiency in general refers solely to the adverse effect of increased risk for infection. Immunosuppression can be deliberately induced in certain health conditions in order to achieve positive results.

2.2 Deliberately induced immunosuppression

Administration of immunosuppressive drugs or immunosuppressants is the main method of deliberately induced immunosuppression. In optimal circumstances, immunosuppressive drugs are targeted only at any hyperactive component of the

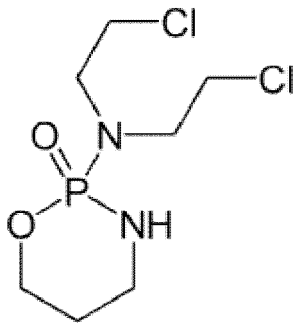
immune system (Starzl *et al*, 1993), and in ideal circumstances would not cause any significant immunodefficiency. However, in essence, all immunosuppressive drugs have the potential to cause immunodeficiency. Immunodeficiency may manifest as increased susceptibility to opportunistic infections and decreased cancer immunosurveillance. The term immunotoxins is also sometimes used (incorrectly) to label undesirable immunosuppressants, such as various pollutants. Polychlorinated biphenyls (PCB) and the insecticide DDT are immunosuppressants. Immunosuppressants may be prescribed when a normal immune response is undesirable, such as in autoimmune diseases.

Cortisone was the first immunosuppressant identified, but its wide range of side-effects limited its use. The more specific azathioprine was identified in 1959, but it was the discovery of cyclosporine in 1970 that allowed for significant expansion of kidney transplantation to less well-matched donor-recipient pairs as well as broad application of liver transplantation (Kautman *et al*, 1995), lung transplantation, pancreas transplantation (Lenschow *et al*, 1992), and heart transplantation (Isobe *et al*, 1992). After organ transplantation, the body will nearly always reject the new organ(s) due to differences in human leukocyte antigen haplotypes between the donor and recipient (Colvin, 1990). As a result, the immune system detects the new tissue as ñhostileò, and attempts to remove it by attacking it with recipient leukocytes, resulting in the death of the tissue (Ferrara *et al*, 1991). Immunosuppressants are applied as a countermeasure; the side-effect is that the body becomes more vulnerable to infections and malignancy, much like in an advanced HIV infection.

Throughout its history, radiation therapy has been used to decrease the strength of the immune system. In general, deliberately induced immunosuppression is performed to prevent the body from rejecting an organ transplant, treating graft-

versus-host disease after a bone marrow transplant (Sigal and Dumont, 1992), or for the treatment of auto-immune disease such as rheumatoid arthritis or Crohnôs disease. This is typically done using drugs, but may involve surgery, plasmapheresis, or radiation. A person who is undergoing immunosuppression, or whose immune system is weak for other reasons (for example chemotherapy and HIV) is said to be immunocompromised. An **immunosuppressant** is any agent that causes immunosuppression, including immunosuppressive drugs and some environmental toxins. Immunosuppression can also be induced deliberately for research purposes to assess the effects of some drugs or herbs, using experimental animals.

2.3 Cyclophosphamide



GENERIC NAME: CYCLOPHOSPHAMIDE

BRAND NAME: CYTOXAN

TRADE NAME lyophilizedcytoxane

PHARMACOKINETIC DATA

Bioavailability	>75% oral
Protein binding	>60%
Metabolism	hepatic

Half life	3-12 hours
Excretion	renal
Chemical formula	$C_7H_{15}Cl_2N_2O_2P$

Cyclophosphamide (Endoxan, Cytoxan), also known as cytophosphane, is a nitrogen mustard alkylating agent (Takimoto, *et al* (2005), from the oxazaphosphinans group. cyclophosphamide belong to the group of immunomodulatory drugs that act by covalent alkylation of other molecules. It has no alkylating ability itself but many of its metabolites are active, each having two active sites to effect the cross-linking of DNA strands. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. This interferes with the strand separation during DNA replication by forming intrastrand and interstrand DNA cross-links (Starzl *et al* 1993). Cyclophosphamide is very good for treatment of cancer and autoimmune disorders. As a prodrug, it is converted by liver enzymes cytochrome P450 (CYP) to form the metabolite 4-hydroxy cyclophosphamide that has chemotherapeutic activity (Huttunen *et al*, 2011). Cyclophosphamide has severe and life-threatening adverse effects, including acute myeloid leukemia, bladder cancer, haemorrhagic cystitis, and permanent infertility, especially at higher doses (Kasper, *et al,* 2005).

Medical uses

Cyclophosphamide is an alkylating drug. It is used primarily for the treatment of breast cancer, ovarian cancer, nephritic syndrome in children, leukemia and certain autoimmune disease. It suppresses B-cell, that activate antibody formation. In other to work, cyclophosphamide is first converted by the liver to acrolein and phosphamide. Acrolein and phosphamide are active compounds and they slow the growth of cancer cells by interfering with the action of deoxyribonucleic acid

(DNA) within the cancerous cells. Unfortunately, normal cells are also affected and this result to serious side effects. Therefore itôs major toxic effects is myelosuppression, alopecia, nausea, and vomiting.

Goals of therapy are prompt control of the underlying pathological process and discontinuation or replacement of cyclophosphamide with less toxic, alternative medication as soon as possible in order to minimize associated oxidized morbidity. Renal function require regularly and frequent monitor by laboratory evaluations, avoid drug-induced bladder complications, and screen for bone marrow toxicity. Like other alkylating agents, cyclophosphamide is teratogenic and should not be used for pregnant women (pregnancy category D) except for life-threatening circumstances in the mother (Clowse *et al* 2005). Cyclophosphamide is not administered during pregnancy. Use of cyclophosphamide during pregnancy may affect the fetus. Fetus exposed to cyclophosphamide may be born with missing fingers, toes, and poorly developed heart (Vaux *et al*, 2003). Additional relative contraindications to the use of cyclophosphamide include lactation, active infection, neutropenia, or bladder toxicity.

Cancer

The main use of cyclophosphamide is with other chemotherapy agents in the treatment of lymphomas, some forms of brain cancer, leukemia (Shanafelt, *et al* 2007), and some solid tumors (Young *et al*, 2006). It is a chemotherapy drug that works by inducing the death of certain T cells. A study showed that the biological actions of cyclophosphamide are dose-dependent. At higher doses, it is associated with increased cytotoxicity and immunosuppresion, while at low continuous doses, it shows immunostimulatory and antiangiogenic properties (Nicolini *et al* 2004). A 2009 study of 17 patients with docetaxel-resistant hormone refractory prostate cancer showed a prostate-specific antigen (PSA) decrease in 9 of the 17 patients.

Median survival was 24 months for the entire group, and 60 months for those with a PSA response. The study concluded low-dose cyclophosphamide ñmight be a viable alternativeò treatment for immunotherapy, tyrosine kinase inhibitors, and antiangiogenisis (Nelius *et al*, 2010).

Autoimmune diseases

Cyclophosphamide decreases the immune system response, and although concerns about toxicity restrict its use to patients with severe disease, it remained an important treatment for life-threatening manifestations of autoimmune diseases where disease-modifying antirheumatic drugs (DMARDs) have been ineffective. For example, systemic lupus erythematosus with severe lupus nephritis (Steinberg *et al*, 1971), may respond to pulsed cyclophosphamide. Cyclophosphamide is also used to treat minimal change disease, severe rheumatoid arthritis, (Townes *et al*, 1976), Wegenerôs granulomatosis (Novack and Pearson, 1971) and multiple sclerosis (Makhani *et al*, 2009).

Side effects of cyclophosphamide

Adverse drug reactions from cyclophosphamide include chemotherapy induced nausea and vomiting, (Singh *et al*, 1991) bone marrow suppression, (Lohrmann, 1984). Stomach ache, hemorrhagic cystitis, diarrhea, darkening of the skin/nails, alopecia (hair loss) or thinning of hair, changes in color and texture of the hair, and lethargy. Other side effects may include easy bruising/bleeding, joint pain, mouth sores, slow-healing of existing wounds, unusual decrease in the amount of urine, or unusual tiredness or weakness. Cyclophosphamide is carcinogenic and may increase the risk of developing lymphoma, leukemia, skin cancer, transitional cell carcinoma of the bladder or other malignancies (Bernatsky *et al*, 2008). Myelo-proliferative neoplasm, including acute leukemia, non-hodgkin lymphoma, and

multiple myeloma, occurred in 5 of 119 rheumatoid arthritis patients within the first decade after receiving cyclophosphamide, compared with one case of chronic lymphocytic leukemia in 119 rheumatoid arthritis patients without a history of cyclophosphamide use (Radis *et al.* 1995). Secondary acute myeloid leukemia (therapy-related AML or t-AMLô) is thought to occur either by cyclophosphamide inducing mutations or selecting for a high-risk myeloid clone (Larson, 2007). This risk may be dependent on dose and a number of other factors, including the condition being treated, other agents or treatment modalities used (including radiotherapy), treatment intensity and length of treatment. For some therapeutic course of treatment, it is a very rare occurrence. For instance, CMF-therapy for breast cancer (where the cumulative dose is typically less than 20 grams of cyclophosphamide) seems to carry an AML risk of less than 1/2000th, with some studies even finding no increased risk compared to the background population. Other treatment regimens involving higher doses may carry risks of 1-2% or higher, depending on regimen. Cyclophosphamide-induced AML, when it happens, typically reoccur some years after treatment, with incidence peaking around 3ï 9 years. After nine years, the risk has fallen to the level of the regular population. When AML occurs, it is often preceded by a myelodysplastic syndrome phase, before developing into overt acute leukemia. Cyclophosphamide-induced leukemia will often involve complexe cytogenetics, which carries a worse prognosis than *de novo* AML.

Emadi *et al*, 2009 revealed that acrolein is toxic to the bladder epithelium and can lead to hemorrhagic cystitis, which is associated with microscopic or gross hematuria and occasionally dysuria. Risks of hemorrhagic cystitis can be minimized with adequate fluid intake, avoidance of nighttime dosage, and mensa (sodium 2-mercaptoethane sulfonate), a sulfhydryl donor which binds and

detoxifies acrolein (Monach *et al*, 2010). Intermittent dosing of cyclophosphamide decreases cumulative drug dose, reduces bladder exposure to acrolein, and has equal efficacy to daily treatment in the management of lupus nephritis. Cyclophosphamide has been found to significantly increase the risk of premature menopause in female and of infertility in male and female alike, the likelihood of which increases with cumulative drug dose and increasing patient age. Such infertility is usually temporary but can rarely be permanent (Balow *et al*, 1987). The use of leuprolide in women of reproductive age before administration of intermittently dosed cyclophosphamide may diminish the risks of premature menopause and infertility (Periti *et al*, 2002). Cyclophosphamide is a pregnancy category D drug and has been shown to cause birth defects. First trimester exposure to cyclophosphamide for the treatment of cancer or lupus has shown a pattern of anomalies labeled ñcyclophosphamide embryopathy,ò including growth restrictions, ear and facial abnormalities, absence of digits, and hypoplastic limbs (Vaux *et al*, 2003). Women previously treated with alkylating agents are often able to conceive and deliver healthy children (Huong, *et al.* 2002). Neutropenia or lymphoma arising secondary to cyclophosphamide usage can predispose patients to bacterial, fungal and opportunistic infections (Pryor *et al*, 1996). There are no published guidelines for pneumocystis pneumonia prophylaxis for patients with rhuematological disease receiving immunosuppressive drugs, but some advocate its use when receiving high-dose medication (Suryaprasad and Stone, 2008). Pulmonary injury appears rare, (Twohig and Matthay, 1990), but can present with an early, acute pneumonitis and a chronic, progressive fibrosis (Malik *et al*, 1996). Cardiotoxicity is a major problem with oncology patients treated with higher dose regimens (Floyd *et al*, 2005). High-dose intravenous cyclophosphamide can also cause the syndrome of inappropriate anti-diuretic hormone secretion (SIADH) and a potentially fatal hyponatremia when compounded by the intravenous fluids

administered to prevent drug-induced cystitis (Bressler and Huston, 1985). While SIADH has been described primarily with higher doses of cyclophosphamide, it can also occur with the lower doses used in the management of inflammatory disorders (Salido *et al*, (2003).

Pharmacology

Oral cyclophosphamide is rapidly absorbed and then converted by mixed-function oxidase enzyme (cytochrom P450 system) in the liver to active metabolites (Cohen and Jao, 1970). The main active metabolite is 4-hydroxycyclophosphamide, which exists in equilibrium with its tautomer, aldophosphamide. Most of the aldophosphamide is then oxidized by the enzyme aldehyde dehydrogenase (ALDH) to make carboxycyclophosphamide. A small proportion of aldophosphamide freely diffuses into cells, where it is decomposed into phosphoramide mustard and acrolein (Boddy and Yule, 2000). Wiernik Duncan, 1971 discovered that active metabolites of cyclophosphamide are highly protein bound and distributed to all tissues, are assumed to cross the placenta and are known to be present in breast milk. Cyclophosphamide metabolites are primarily excreted in the urine unchanged, and drug dosing should be appropriately adjusted in the setting of renal dysfunction (Haubitz *et al,* 2002). Drugs altering hepatic microsomal enzyme activity (e.g., alcohol, barbiturates, rifampin, or phenytoin) may result in accelerated metabolism of cyclophosphamide into its active metabolites, increasing both pharmacologic and toxic effects of the drug; alternatively, (corticosteroids, tricyclic antidepressants, or allopurinol) that inhibit hepatic microsomal enzymes result in slower conversion of cyclophosphamide into its metabolites and consequently reduced therapeutic and toxic effects (Donelli, *et al.* 1976). Cyclophosphamide reduces plasma pseudocholinesterase activity and may result in prolonged neuromuscular blockade when administered concurrently

with succinylcholine (Koseoglu *et al*, 1999). Tricyclic antidepressant and other anti-cholinegic agents can cause delayed bladder emptying and prolonged bladder exposure to acrolein.

Mechanism of action

The main effect of cyclophosphamide is due to its metabolite phosphoramide mustard. This metabolite is only formed in cells that have low levels of ALDH (Aldehyde dehydrogenase). Phosphoramide mustard forms DNA crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand crosslinkages, respectively). This is irreversible and leads to cell apoptosis (Hall and Tilby, 1992). Cyclophosphamide has little typical chemotherapy toxicity as ALDHs are present in relatively large concentrations in bone marrow stem cells, liver and intestinal epithelium. ALDHs protect these actively proliferating tissues against toxic effects of phosphoramide mustard and acrolein by converting aldophosphamide to carboxyphosphamide that does not give rise to the toxic metabolites phosphoramide mustard and acrolein. Cyclophosphamide induces beneficial immunomodulatory effects in adaptive immunotherapy. Suggested mechanisms include (Sistigu *et al*, 2011),

Elimination of T regulatory cells (CD4+CD25+ T cells) in naive and tumor-bearing hosts

Induction of T cell growth factors, such as type I IFNs, and/or

Enhanced grafting of adoptively transferred, tumor-reactive effector T cells by the creation of an immunologic space niche.

Thus, cyclophosphamide preconditioning of recipient hosts (for donor T cells) has been used to enhance immunity in naïve hosts, and to enhance adoptive T cell immunotherapy regimens, as well as active vaccination strategies, inducing objective antitumor immunity.

2.4 BLOOD

Blood is a circulating tissue of the body, it consists of a pale yellow fluid, plasma in which are suspended red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes) (Stedmam2006), blood delivers oxygen, hormones, and nutrients to the body, removes waste products from the body and act as primary carrier of immunity (Dailey, 2002).

HAEMOPOIESIS

Haemopoiesis is the process of formation and development of various blood cells and other formed elements (Stademan 2006). Haemopoiesis also refer to the production of blood cells in the bone marrow.

Site of haemopoiesis: All blood cells develop from the immature and undifferentiated cells called a stem cell that resides in the bone marrow. Many different conditions control the productions of blood cells. For example low oxygen in the body tissues stimulates red cell (erythrocyte) production. Infection causes an increase in white cell (leucocytes) production, while blood loss enhances platelet (thrombocyte) production. Blood cell development occurs in different sites at various stages of human development (Lapidot *et al* 2005). From the third week of fetal growth amniotic sac develops and within this sac stem cells form clusters called blood-island. These blood islands are the precursors of blood cells and blood vessels of the vascular system. From the third month of fetal growth, blood cell production moves from the amniotic sac to the liver. Thereafter during this month blood cell development takes place in the spleen and thymus. By the fourth month, bone marrow produces all cell lines and continues to do so throughout life

(Taichman, 2005). At birth almost all bones of the body produce blood cells. By adolescence only the flat bones like skull, sternum, vertebrae, ribs and pelvis are involved in blood cell development. In adults the ribs, sternum, vertebrae, and pelvis produce blood cells.

Haemopoietic stem cells (HSC): Haemopoiesis is derived from a population of stem cells, which starts with a pluripotential stem cells in the bone marrow and give rise to the separate cell lineages (Hoffbrand *et al* 2006). Cell differentiation occurs from the stem cells via the haemopoietic progenitors which are restricted in their developmental potentials. The existence of the separate progenitor cells can be demonstrated using in vitro techniques. The bone marrow is also the primary site of origin of lymphocytes which differentiate from a common lymphoid precursor. One stem cell is capable of producing about 10^6 mature blood cells. The precursor cells are capable of responding to haemopoietic growth factors with increased production of one or other cell lines if the need arises (Orkin and Zon,

2002).

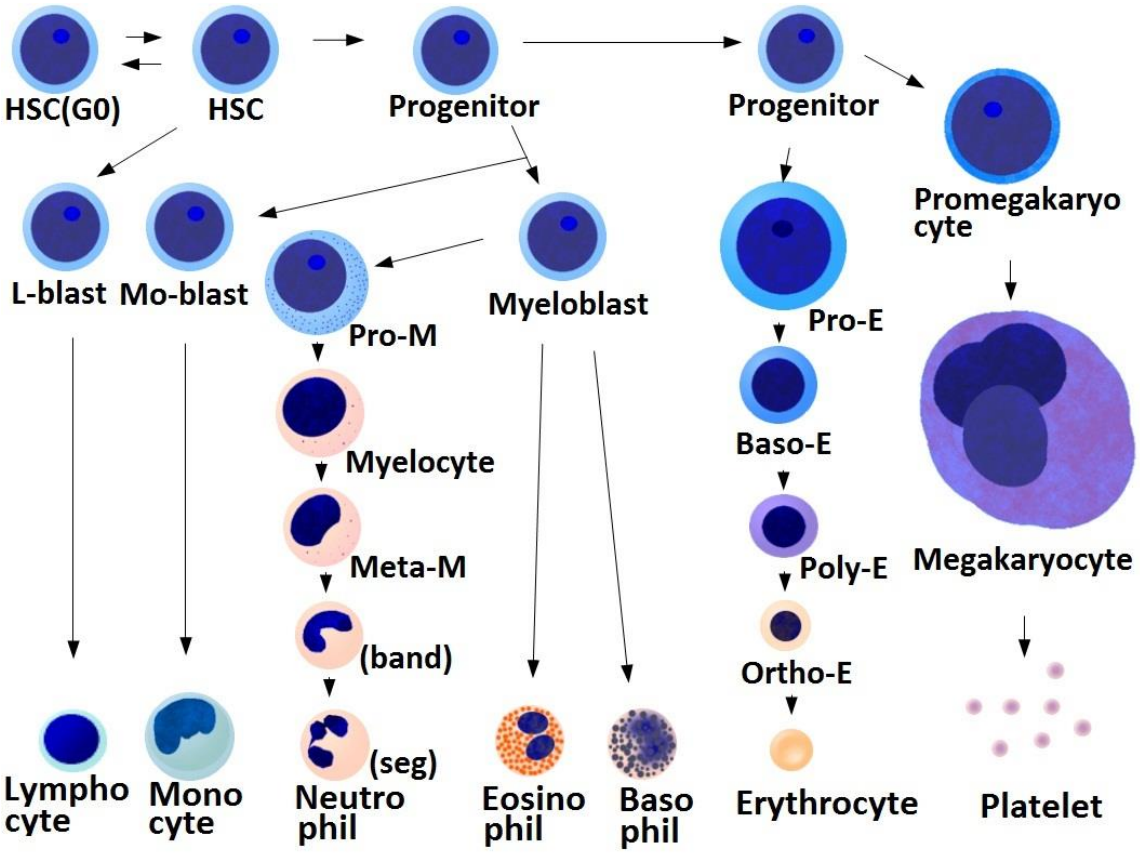


Fig 1. Pluripotent stem cell and cell lines.

Fig 1.1, is a diagrammatic representation of bone marrow pluripotent stem cell and cell line that arise from it. Various progenitors can be identified by culture in semi-solid medium by the type of colony they form. basophil , burst forming unit; CFU, colony forming unit: Erythroid; Eosinophil; neutrophil, monocyte megakaryocyte; and NK, natural killer cells.

TYPES OF BLOOD CELLS

There are three types of blood cells: red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocyte). Erythrocytes are produced by the process of erythropoiesis which pass from the stem cell through the progenitor cells colony-forming unit granulocyte, erythroid, monocyte,and megakaryocyte

(UFU_{GEMM}), Burst forming unit erythroid (BFU_E) and erythroid (CFU_E) (Cantor, 2002). Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes one of the largest cell of the body, while leucocytes are produced by the process of leucopoiesis. Each kind of blood cell has a unique function.

Erythrocytes (Hemoglobin): erythrocytes transport oxygen from the lung to body cells and help transport carbon dioxide from body cells to the lungs.

Platelets (thrombocytes): Platelets are essential in preventing blood loss by forming a plug at the site of vascular injury.

Leucocytes: Leucocytes (white blood cells) are part of the immune system, which protect the body against microorganisms and foreign invaders. The white blood cells are divided into phagocytes and immunocytes. Granulocytes include neutrophils, eosinophils and basophils together with monocytes are the phagocytes. Only mature phagocytes and lymphocytes are found in normal peripheral blood. The function of phagocytes and immunocytes in protecting the body against infection is closely connected with two soluble proteins of the body: immunoglobulin and complement.

Neutrophils (polymorphs): Neutrophils are produced in the bone marrow, the earliest recognizable precursor is the myeloblast which give rise to promylocyte with primary granules in the cytoplasm, this then produce myelocytes, metamyelocytes (with primary and secondary granules) then the band cells which may occur in the peripheral blood, and then the mature neutrophils with characteristic dense nucleus consisting of between two to five lobes and a pale cytoplasm with an irregular out line containing many fine pink-blue or grey- blue granules. The granules are divided into primary, which appear at the promyelocyte stage and secondary which appear at the myelocyte stage and predominate in the

mature neutrophil. Both types of granules are lysosomal in origin, the primary contains myeloperoxidase, acid phosphatase and other acid hydrolase; the secondary contain collagenase, lactoferrin and lysozyme (Hoffbrand *et al*, 2006). Neutrophils are the main defence of the body against pyogenic bacteria antigen (Dacie and Lewis 1994), neutrophils act quickly in vast number in response to inflammation and infections, they are strongly phargocytic, their membrane has Fc, C3 receptor that bind antibody and complement which enhances phargocytosis (Dailey, 2001).

Monocytes: Monocytes are usually larger than other peripheral blood leucocytes and possess a large central oval or indented nucleus with clumped chromatin. The abundant cytoplasm stained blue and contains many fine vacuoles, giving a ground glass appearance. Monocytes spend only a short time in the bone marrow and after circulating for 20-40 hours, leaves blood and enters the tissues where they mature and transform into macrophages, they may assume specific function in deferent tissues (e.g. skin, liver, spleen, gut, etc). One of the monocyte lineages is dendritic cells which are involved in antigen presentation to T cells. GM-CSF (granulocyte-macrophage colony stimulating factor) and M-CSF (monocyte-colony stimulating factor) are involved in their production and activation (Hoffbrand *et al*, 2006).

Eosinophils: Eosinophil and neutrophil are similar accept that the cytoplasmic granules of eosinophil is coarser and more deeply red stained and rarely more than three nuclear lobes. Eosinophils enter inflammatory exudates and play a role in allergic response, defence against parasites and removal of fibrin formed during inflammation.

Basophils; these are only seen occasionally in normal peripheral blood. They have many dark cytoplasmic granules which overlie the nucleus and contain histamine

and heparin. In the tissue they become mast cells. They have immunoglobulin E (IgE) attachment site and their granulation is associated with histamine release. Following their release from bone marrow granulocytes spend only 6-10 hours in the circulation before moving into tissues where they perform their phagocytic function.

Lymphocytes:

Lymphocytes are the immunological competent cells that assist the phagocytes in defence of the body against infections and other foreign invasions. Two unique features of the immune system are the ability to generate antigenic specificity and the phenomenon of immunological memory. In post natal life, the bone marrow and thymus are the primary lymphoid organs in which lymphocytes develop. The secondary lymphoid organs in which specific immune responses are generated are the lymph nodes, the spleen, and lymphoid tissues of the alimentary and respiratory tracts (Randulph *et al*, 2005). The immune response depends upon two types of lymphocytes, B and T cells, which is derived from the haemopoietic stem cells. B cells mature in the bone marrow and circulate in the peripheral blood until they undergo recognition of antigen. The B-cell receptor is membrane-bound immunoglobulin and after activation it is secreted as free soluble immunoglobulin. At this point they mature into memory B cells or plasma cells. T cells develop from cells that have migrated to the thymus where they differentiated into mature T cells during passage from the cortex to the medulla. During this process, self-reactive T cells are deleted (negative selection) whereas T cells with some specificity for host human leucocytes antigen (HLA) molecules are selected (positive selection). The mature helper cells express CD4 and cytotoxic cells expresses CD8. The cells also expresses one of two T cells antigen receptor heterodimers, Ŭƃ (>90%) or ɔŭ (<10%), and recognizes antigen only when it is

presented at the cell surface (Hsu, 2005). Lymphocytes provide a specific immune response when antigen invades the body. They are activated when they recognize foreign matter as non self. Lymphocytes make up about 25% of circulating white blood cells some lymphocytes released from the hematopoietic marrow circulate in the blood and others migrate to lymph nodes where they wait for antigens (Dailey, 2002). Lymphocytes play a role in the bodyôs rejection of transplanted organs. Host lymphocytes recognize antigens on the donor organ as foreign. Host lymphocytes attack and destroy foreign tissue in what is referred to as organ rejection, or host-versus-graft disease.

Natural killer cells: Natural killer (Nk) cells are cytotoxic $CD8^+$ cells that lack the T-cell receptor (TCR). They are large cells with cytoplasmic granules and typical express surface molecules CD16 (Fc receptor), CD 56 and CD57. NK cells are designed to kill target cells that have a low level of expression of HLA class 1 molecule such as may occur during viral infection or on a malignant cell (Hoffbrand *et al*, 2006).

2.5 OCIMUM TENUIFLORUM (NCHUANWU) LEAF.

Ocimum tenuiflorum is a perennial herb. Scientifically classified as follows:

Kingdom:	Plantae
Unranked:	Angiosperms
Unranked:	Eudicots
Unranked:	Astrerids
Order :	Lamieles
Family:	Lamieceae
Genus:	*Ocimum*
Species :	*Ocimum tenuiflorum*
Binomial name:	*Ocimum tenuiflorum*

(Pattanayak *et al* 2010)

BOTANICAL CLASSIFICATION

Ocimmum tenuiflorum leaf is a latin word meaning (ñbasil with small flower.óò It is also called *Ocimum sanctum* (óósacred frangrant lipped basilóò) or *Ocimum gratissimum* (óóvery grateful basilóò) (Steven 2004). *Ocimum tenuiflorum* is also called *tulsi* in India, holy basil in English, while Igbos in Eastern Nigeria called *Ocimum "Nchuanwu, Ahigbe or Alili*ò. It is a scent leaf in the family of lamieceae which is found throughout the world, because, it is widely cultivated (staple *et al*, 1999). It is an erect branched sub-shrub about 30-60cm tall with hairy stem, and very penetrating root. The leaves are strongly aromatic (Kothari *et al,* 2005). The leaves have petioles and are oval in shape, about 5cm tall, and slightly toothed. The flowers have three major colours, green, dark green, and purple (Steven 2004) Two main colours are cultivated in India, green-leafed (lakshmi tulsi) and Krishna (*tulsi*) (Kothari and Bhattachary *et al,* 2005). They are mainly cultivated for religious and medicinal purposes (Warrier, 1995) and for its essential oil (Kelm *et al*, 2000). Throughout south Asia this leaf is known as medicinal plant and an herbal tea, commonly used in ayurveda (staple *et al,* 1999). *Ocimum tenuiflorum* has an important role within the vaishnavas tradition of Hinduism in which devotees worship the *tulsi* plant or the leaves (Flood, 2001). There are various types that belong to *Ocimum* family, their chemical composition changes according to seasons and are affected by different soil, harvesting, processing, and storage conditions (Steven, 2004).

NUTRITIONAL COMPOUND

The nutritional component of *Ocimum tenuiflorum* include: Vitamins A and C; Minerals: calcium, iron, and zinc, as well as chlorophyll (Anbarasu *et al*, 2007).

2.6 ACTIVE COMPOUDS OF *OCIMUM TENUIFLORUM*

The leading phytochemical compounds of *Ocimum tenuiflorum* leaf include: Alkaloid, Glycoside, Saponin, Tannin, Flavonoid, resin, Protein, Oil, Steroids, Reducing sugar, Terpenoid, carbohydrate, and acidic compound. Other active compounds according to Silva *et al,* are; eugenol (volatile oil), ursolic acid (triterpenoid), and rosemerinic acid (phenyl propanoid). B-caryophyllene and oleonolic acid (Silva *et al*, 2010). The seeds contain fixed oil, linoleic acid and linolenic acid. *Ocimum tenuiflorum* does not contain caffeine or other stimulants (Steven, 2004).

2.7 MEDICINAL USES OF *OCIMUM TENUIFLORUM*

It has been reported that *Ocimum tenuiflorum* has been used for so many years in Indian system of healing called ayurveda due to its medicinal values (NIIR Board, 2004). *Ocimum* is used for treatment of bronchial asthma, it acts as bronchial dialator, and possess anti cancer, anti oxidant, anti asthmatic, anti-inflammatory, anti helminthic, anti diabetic, anti bacterial and anti viral properties. It protects the body from radiation poisoning and cataract, *Ocimum tenuiflorum* is a good herb for treatment of common cold, fever, and malaria. It has cardioprotective, hepatoprotective, hypotensive, hypolipidemic and immunomodulatory effects (Sai, *et al,* 2014). *Ocimum* in herbal medicine is a natural substance considered to help the body adapt to stress (Maity *et al*, 2000), it is an (adaptogen) (Kuhn *et al*, 2007). It is used in many homes for wound healing, it is also used for the treatment of the following ailments; genitourinary disorders, digestive disorders, skin diseases, various forms of poisoning, diuretic, diarrhea and arthritis (Sai, *et al*, 2014). *Ocimum* is believed to increase longevity (puri *et al*, 2002). Recent study suggests that tulsi (*Ocimum*) may be a cox-2 inhibitor, due to its high concentration of eugenol (1-hydroxy-2-methoxy-4-allybenzene) (Prakash and Gupta, 2005). One study showed *tulsi* to be very helpful in the treatment of ailments and many serious

systemic diseases as well as localized infections (Gupta *et al*, 2002). This could be due to the presence of tannin and flavonoid that has antibacterial and antiviral properties.

2.8 DIGESTIVE SYSTEM

A study showcased *tulsi* as anti- diabetic (Rai *et al*, 1997). It is very effective for the treatment of diabetes by reducing blood glucose level, due to its anti oxidant properties (Sethi *et al* 2004). The same study showed significant reduction in total cholesterol level with *tulsi* (Rai *et al*, 1997). The anti oxidant could be as a result of flavonoid, and tannin content of the leaf. The fixed oil demonstrate anti-hyper lipidemic and cardio protective effects in rats fed with a high fat diet due to inhibition of lipid per oxidation activity(Suanarunsawat *et al,* 2010). Steroid has the ability to reduce elevated cholesterol while flavonoid modifies blood lipid level, regulate carbohydrate and glucose metabolism, and also protects intestinal mucosa. *Ocimum tenuiflorum* contributes to healthy liver function (Arhoghro *et al,* 2009) and counteracts various liver diseases (Sai *et al*, 2014). Ethanol extracts showed hepato protective effect **(**Surana and Jain, 2010), it protects the liver and improves the metabolic breakdown and elimination of dangerous chemicals in the body (detoxification) (Akilavalli *et al* 2011). *Ocimum tenuiflorum* balances blood sugar and insulin metabolism, can reduce fasting blood glucose (Rai *et al*, 1997). The essential oil relaxes the muscle of the small intestine (Socorro *et al*, 2002). Igbos in eastern Nigeria use *Ocimum* to treat stomach cramp by cooking yam porridge with *Ocimum tenuiflorum,* or by chewing the fresh leaves.

A study reveals that *Ocimum tenuiflorum* improves the digestive system (Geeta *et al*, 2001) such as constipation (especially when its tea is taken with dried ginger), heart burn, bloating, vomiting, intestinal gas, diarrhea dysentery, intestinal worm remover when the fresh juice is taken with honey (Steven, 2004), it inhibits the

growth of E.coli and reduces bad breath (Veronica *et al*, 1999). The intestinal worm removal was also confirmed by our experiment, about two of our experimental rats passed out a long white worm, during the *Ocimum* extract administration.

2.9 WOUND HEALING.

Tulsi is found to be anti-ulcerogenic (Dharmani *et al*, 2004) as well as ulcer healing properties and could act as therapeutic agent against peptic ulcer diseases; it decreases incidence of gastric ulcer (Steven, 2004). It reduces the effect of irritating drugs on the stomach lining and increases the production of protective stomach mucous. Souza *et al* discovered that tannin isolated from the stem bark also has anti-inflammatory and antiulcer activity in rodents, (Souza, *et al,* 2006). In Eastern part of Nigeria, fluid squeezed out from fresh *Ocimum* leaf is used to treat small wound.

2.10 CARDIOVASCULAR CIRCULATORY SYSTEM

*Tulsi i*s an excellent heart tonics, it prevents heart attacks, lowers stress-related high blood pressure and protects the body against damage caused by foreign toxins in the blood (Sai *et al,* 2014). Study showed that drinking of *tulsi* leaf tea keeps the blood pressure balanced (Sharm *et al*, 2001). Clinical studies were undertaken in case of bronchial asthma and found to be effective in management of asthma and even immunological disorder including allergies, viral encephalitis, stress related arterial hypertension (Steven, 2004). It protects components of heart and liver cells from oxidative damage caused by free radicals generated by chemotherapy (Surana and Jain, 2010). *Ocimum* (*tulsi*) have high concentration of alkaloid, glycoside, and saponin; alkaloid is anti high blood pressure, cardiac stimulator, and reduces bronchial asthma (Hesse, (2002). A study by David et al showed that combination of glycoside and saponin produces wonderful effects in medicine. Saponins are

called cardiac glycosides because they modify heart actions. Cardenolides inhibit the Na^+-K^+-ATPase pump in mammals. Several plants with cardiac glycosides are used medicinally. These plants are often used to treat heart problems. (David *et al* 2014).

2.11 IMMUNE SYSTEM

A study revealed that *Ocimum tenuiflorum* or *tulsi* strengthens and regulates the immune system (Singh *et al*, 2007), enhances humoral and cell mediated immunity. It increases T-cell activity. Our hematological analysis is in agreement with this literature based on our results, the suppressed immunity later increased following the administration of *Ocimum tenuiflorum* extract by oral route. Probably due to the presence saponin and glycoside, combination of the two used as adjuvant in vaccine stimulate both the Th1 immune response and the production of cytotoxin (CTLs) against exogenous antigens, (Sun *et al,* 2009).

Anti-inflammatory action: It reduces the painful and dangerous inflammation that plays a key role in various forms of arthritis, cancer and degenerative neurological disorders. *Ocimum tenuiflorum* is useful in compounded state of the humoral and cellular immune system (Rajendra, 2013). It regulates the óóstressôô responses, helps the body adapt to stress (Ashwani, 2012), supports normal body functions and restores balance. It also has anti viral activities, anti leukemia, anti tumor and anti cancer properties (Nerenda *et al*, 2002). Flavonoids reduce oxidative stress, modifies intracellular signaling pathway in immune cells and acts as anti inflammatory by inhibiting reactive oxygen and nitrogen compounds, (Izzi *et al,* 2012). Flavonoid, saponin, and alkaloids have anti cancer activities. flavonoid intake is associated with reduced gastric carcinoma risk in women (González *et al* 2013). *Ocimum tenuiflorum* extract have been reported to act like aspirin and ibuprofen, but it is not irritating to the stomach. It has immune stimulant properties

because it inhibits antigen induced (allergic) histamine. Reduces asthmatic and other adverse immune reactions in drugs used for the treatment of cold and flu (Mondal *et al,* 2011). Anti-ageing:- supports the body and mind in reducing the negative effect of ageing. Relieves canker sores and useful in pyorrhea (gum infection). *Ocimum tenuiflorum* has the ability to increase bodyôs resistance to stress by stimulating a non-specific, self regulation in adapting to stress (Ashwani, 2012). This could be as a result of alkaloid, glycoside, and flavonoid content of *Ocimum.*

2.12 SKIN

Ocimum tenuiflorum juice is very good for the treatment of ringworm and eczema, ache, and pimples, it has been found successful in the treatment of leucoderma (Rajendra, 2013). *Ocimum T.* reduces psoriasis and various difficult skin disorders like leprosy and staph infection of the skin. Another study states that *Ocimum tenuiflorum (tulsi)* beneficial effect is its anti oxidant properties, it provides skin anti oxidant and free radical scavenging protection therefore reducing oxidative stress and cell damage due to the anti oxidant activities(Devi *et al*, 1999). This could be the effect of flavonoid, glycoside, saponin and tannin.

Sharma reported that *tulsi* also has the ability to protect the DNA of the body from the dangerous mutating power of various forms of radiation poisoning and cataracts (Sharma *et al* 1998). Essential oil is also used in herbal skin cosmetics due to its antibacterial and antifungal activities (Uma, 2001). It contains ursolic acid which is one of the cosmetics industryôs latest favorites because not only does it quickly heal skin, return elasticity and removes wrinkles, but it is also good at preventing and curing skin cancer (Rajendra, 2013). *Ocimum tenuiflorum* paste mixed with black paper is very useful for treatment of ringworm, eczema (Ashwani, 2012), the paste was found to reduce insect bites (Oparaocha *et al*,

2010). Tannin, terpenoid and resin have been reported to be pesticides they produce certain scent that derive pastes away.

Ocimum tenuiflorum enhances protein synthesis, muscle mass and strength (Steven, 2004). It contains vitamin A and is a very good remedy for sore eyes and night blindness, and to treat conjunctivitis (Rajendra, 2013). The juice of *Ocimum tenuiflorum* mixed with honey is used as an eye wash to either wash the eyes or spread on the tender skin below the eyes (Sai *et al*, 2014). *Ocimum* contain proteins which are involved in all aspect of chemical reactions in the body, they perform great functions within living organisms by acting as enzymes.

2.13 NERVOUS SYSTEM

Ocimum tenuiflorum can stop convulsion (Sai *et al*, 2014). It quickens the transmission of nerve impulses from the central nervous system to outside, to bring about activity in a muscle or gland. It brings to normal the neurotransmitter in the brain and keeps the neurochemistry in the brain in a state similar to anti depressant medication. It sharpens memory. Its oil serves as pain reliever to the ear. Fresh garlic juice mixed with cooked *tulsi* in mustard oil and the warm oil placed in the ears stops ear aches (Steven, 2004). Preparations of plants containing alkaloids and their extracts have been used as psychoactive substances. Cocaine, caffeine and cathinone are stimulants of central nervous system, (Veselovskaya *et al* 2000)

2.14 REPRODUCTIVE SYSTEM

Ocimum tenuiflorum was said to have anti fertility effect (Obianime *et al*, 2010). It was suspected to reduce the estrogen hormone levels in female and decreases sperm count in men (Steven, 2004). Large dose of saponin is toxic to the body, it lyses red blood cells and is used as contraceptive drugs (Asl *et al*, 2008). A

particular research found out that *Ocimum tenuiflorum* causes infertility in male mouse, but ethanol extract showed sustained increase in the sexual activity of normal male mice without adverse effects and so it is used traditionally to treat sexual disorders (Pande, 2009).

2.15 RESPIRETORY SYSTEM

Ocimum tenuiflorum contributes generally to respiratory health, supports healthy pulmonary function, provide lung and bronchial support. It is very helpful in the treatment of variety of serious allergies, inflammatory and infectious disorders affecting the lungs and related tissues. It has bronchodilator effects therefore very good for treatment of bronchial asthma. It is used against bronchitis and tuberculosis. It is used for rhinitis (inflammation of nasal mucous membrane) (Ralph *et al,* 2002).

The root of the *Ocimum tenuiflorum* plant crushed and boiled with turmeric powder for few minutes and filtered, two spoonful of this potion twice daily will cure Acute Respiratory Syndrome (SARS) and prevent contracting of the diseases (Das *et al,* 2006). The juice of the leaves is given in catarrh and bronchitis in children. Chewing the leaves relieves cold and flu. A decoction of the leaves, cloves and common salt also give immediate relieve in case of influenza (Ashwani, 2012). Alkaloid function as respiratory stimulant, relieve cold and sinusitis and bronchial asthma (Hesse, 2002), saponin reduce ammonia, and causes less damage to respiratory tract in animals (Zentiner and Edward, 2011), while flavonoid is anti-allergic, (Yamamoto *et al,* 2001), and all are anti-inflammatory.

2.16 URINARY OR EXCRETORY SYSTEM

Seeds of *Ocimum tenuiflorum* are used in curing urinary problems. Seeds are used as diuretics. It has strengthening effect on the kidney. In case of kidney stone,

Ocimum tenuiflorum leaves being a great diuretic and detoxifier, reduces the uric acid levels in the blood (one of the main reason for kidney stones is the presence of excess uric acid in the blood), the presence of acetic acid and other components in its essential oil help in breaking down the kidney stone and its pain killer effect helps to dull down the pain of the kidney stones, the juice if taken regularly with honey for 6months will expel the stone via the urinary tract (Ashwani, 2012).

2.17. PSYCHO-SPIRITUAL

Religiously holy basil as it is called is believed to aid meditation and delivers nutrients to the mind necessary for the experience of enlightenment (Ashwani, 2012). Spiritually empowered and endowed to transform souls. It is believed that basil opens the heart and mind bestowing the energy of love and devotion. It strengthens faith, compassion and clarity (Chatterjee and Gautam, 2001).

In fact it is believed by its devotees that where ever *tulsi* grows is sacred and no misery (Steven 2004). She is the holiest of holy, that where ever the breeze blows her fragrance there is purity. It showers blessing on those who worship and grow *tulsi.* In India, many families keep *tulsi* plant in their homes (Ashwani, 2012), where it is held to create an atmosphere of peace and prosperity. It has long been used for its purifying influences (Flood, 2001). There seems to be many reasons why people use *Ocimum tenuiflorum* .

Most of the research on holy basil has been on animals and very few studies on human. These animal studies tend to corroborate and confirm the relative claims in ayurveda literature (Steven, 2004). Research has confirmed dozens of holy basilôs traditionally known actions and therapeutic uses including its powerful support for the immune system. Observation made by practitioners was that: prolonged period

of time may provide more accurate overall assessment of the effect of holy basil than short term clinical studies and experiments (Steven, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION OF PLANT MATERIAL:

Five kilograms of fresh *Ocimum tenuiflorum* leaves was purchased from a local market in Enugu East local government area and authenticated by taxonomist with the voucher specimen at the herbarium of Department of Plant science and Biotechnology, University of Nigeria Nsukka.

3.2 SAMPLE PREPARATION

The leaves were washed free of sand and other contaminants, it was cut into small pieces, air-dried under a shade for two weeks and ground into powder.

3.3 EXTRACTION OF PLANT MATERIALS

Aqueous and methanol extraction was done using standard procedure of Trease and Evans (1989).

3.4 AQUEOUS EXTRACT:

Four hundred grams of the powder was soaked in 1000ml of distilled water and kept in the refrigerator with periodic shaking of one hour interval for 24 hours, the extract was sieved with muslin cloth, and filtered with what-man number one, water in the filtrate was evaporated at 60^{OC} , the residue weighed 23.65g and was preserved in a refrigerator until needed.

3.5 METHANOL EXTRACT:

Five hundred grams of the leaf powder was soaked in 1000ml of methanol and kept at room temperature (28 ^{OC}) with periodic shaking for 48hours. The mixture was sieved with a muslin cloth and filtered into a stainless bow using what-man

number one and allowed to evaporate at room temperature. The extract obtained weighed 24.23g and was stored in the refrigerator until ready for use.

3.6 ACUTE TOXICITY STUDIES (MEDIAN LETHAL DOSE LD_{50}):

The method of Lorke (1983) was used to investigate the oral dose of *O. tenuiflorum* dried leaf extract that produce immediate or acute toxicity in rats. The oral toxicity testing was conducted separately on the extract, in aqueous and methanol and was in two stages.

This method of acute toxicity makes the following assumptions:

Substances more toxic than 1mg/kg are so highly toxic that it is not important to calculate the LD_{50}.

LD_{50} value greater than 5000mg/kg b.w are of no practical interest.

An approximate figure the LD_{50} is usually adequate to estimate the risk of acute intoxication.

Stage I was an exploratory trials, the animals was divided into 3 groups of 3 rats each. The first group A received 1000mg/kg body weight of the leaf extract. Group (B) received 2000mg/kg body weight. Group C received 3000mg/kg body weight of the extracts by oral root. The animals were constantly observed for 2hours intermittently for the next 4hours and then over night. No dead rats per group occur. The second stage was performed using the following doses; 3500mg/kg, 4500mg/kg, and 5500mg/kg body weight, for 3 groups of rats containing 3 rats per group. The number of dead animals per group was recorded.

3.7 ANIMAL HOUSING

A total of 32 albino rats of mixed sexes aged 2-3 months weighing 150g - 200g were purchased from a disease free animal house. The rats were housed in well ventilated cages in the Animal House of College of Medicine University of Nigeria

Enugu Campus and allowed to acclimatize for 14 days. They were fed daily with commercial pellet diet rat feed and water *ad libitum*.

3.8 EXPERIMENTAL DESIGN

The albino rats were grouped into eight (8) of four (4) rats in each cage.

Group A: Was normal control and were fed with water and commercial pellet rat feed only by oral administration.

Group B: Was cyclophosphamide control group administered with 0.5ml of cyclophosphamide by intra-peritoneal injection and received water plus rat feed once daily for five days. After the drug induction these group were given pellet rat feed only till the end of the research.

Group C: Was administered 0.5ml of cyclophosphamide plus 500mg/kg body weight of aqueous leaf extract once daily by oral rout for 21 days.

Group D: Was administered 0.5ml of cyclophosphamide as in group B and received 1000mg/kg body weight of aqueous leaf extract orally once daily for 21 days.

Group E: Was injected 0.5ml of cyclophosphamide as in group B plus 500mg/kg body of methanol leaf extract orally once daily for 21 days.

Group F: Was administered 0.5ml of cyclophosphamide as in group B and received 1000mg/kg body weight of methanol leaf extract orally once daily for 21 days.

Group G: Received 500mg/kg body weight of aqueous leaf extract plus rat feed and water only for 21 days.

Group H: Received 1000mg/kg body weight of methanol leaf extract, rat feed and water only for 21 days.

All the treatment with the leaf extract was given daily, the experiment lasted for

21days. Blood sample was withdrawn three times during this experimental study. The first sample was drawn on day 6 to ensure that immunosoppression was achieved before treatment commenced. On Days 15 and 22 blood samples was also withdrawn from the animals for analysis to assess the effect of the *Ocimum tenuiflorum* on the immunosuppressed albino rats. At the end of the experimental study the rats were sacrificed and the liver and spleen was used for histological analysis, to determine the effect of oral administration of the leaf extract on the organs.

3.9 BLOOD SAMPLE COLLECTION

2mls of blood sample was withdrawn from the animal through the cantus of the eye, using plain capillary tube. The blood was collected into EDTA (ethylene diamine tetra acetic acid) anticoagulant bottle and was mixed immediately by gentle inversion. The samples were immediately used for analyses of hematological parameters**.**

3.10 BLOOD SAMPLE ANALYSIS

Blood samples were analyzed using an automated cell counter (Mythic 22) five part differential measurement with standard calibration, according to the manufacturerôs instructions for analysis of human blood and accurately programmed for the analysis of hemoglobin concentration (Hb), pack cell volume (pcv), total white blood cell count (wbc), red blood cell count (Rbc), differential leucocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and Platelet counts.

DETECTION PRINCIPLE OF THE AUTOMETED CELL COUNTER

The optical detection system is based on an unique and innovative sample flow and passive sheath. The sample flow is introduced in the flow cell under pressure and

the sheath is dedicated to maintain it. This principle enables to introduce large quantity of sample and to use a great dilution rate which allows doing hemoglobin measurement with the same dilution. For each cell throwing the optical detection area, two pulses are generated, one for the Axis Loss Light measurement and one for the Forward Side Scatter measurement. The result of those two axes of measurement is the high definition matrix that enables to identify five WBC populations. The five part differential is obtained by the optical matrix analysis after action of the lytic reagent. These reagents destroys the RBC and their stroma, composes the oxy hemoglobin chromogen and protect the white blood cell membrane to keep it in close native state.

METHOD

Hemoglobin measurement: The hemoglobin measurement is directly done in the WBC chamber, by spectrophotometry at 555nm. Hemoglobin is detected by formation of a chromogen oxy hemoglobin type (cyanide free technique). A measurement of the blank of hemoglobin is done for each analytic cycle and during the start up rinsing step. An automatic offset circuit for the LED 555nm allows maintaining the blank level at the same range.

Leucocyte Analysis: The leucocyte analysis is done by impedancemetry in the WBC counting chamber. Parameters: Total WBC, Lymphocytes, Monocytes, Neutrophils, Eosinophils, and Basophils, all in percentage.

Erythrocyte Analysis: The erythrocyte analysis is done by impedancemetry in the RBC counting chamber and by analysis of the hemoglobin inside WBC chamber as previously described. Seven parameters are obtained. Parameters: Hemoglobin, Hematocrit. The hematocrit is measured by integration volume of all the red blood cells which flow in the RBC counting chamber aperture.

3.11 STATISTICS

Data were analyzed using two-way analysis of variance (2-WAY ANOVA), with graph pad prism version 5.1. The data were presented as mean ± standard deviation. Probability value (P-value) less than 0.05 were considered statistically significant.

3.12 PHYTOCHEMICAL ANALYSIS OF *OCIMUM TENUIFLORUN* LEAF

These were carried out according to the method described by Trease and Evans. Methanol and Aqueous extract were subjected to qualitative tests for determination of flavonoids, tannins, saponins alkaloids, reducing sugars, Steroids, Terpenoids, Carbohydrate, resins, Proteins, and acid compounds were tested as per standard procedure (Evans, 2006).

3.12.1 ANALYSIS OF CRUDE POWDER OF *OCIMUM TENUIFLORUN* LEAF

METHOD

Test for carbohydrate

About 0.1g of the powdered leaf material was boiled with 2ml of water and filtered. To the filtrate, few drops of napthol solution in ethanol (Molischôs reagent) was added. Concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer.

Observation: A purple interface ring indicates the presence of carbohydrate.

Result: +++

Test for reducing sugars

About 0.1g of the powder was shaken vigorously with 5ml of distilled water and filtered. The filtrate was used in the following tests;

Fehlingôs test: to 1ml portion of the filtrate was added equal volume of fehlingôs solution 1 and 11 and boiled on water bath for few minutes.

Observation: A brick red precipitate, indicate the presence of sugar.

Result: +

Benedictôs test: to 1ml portion of the filtrate was added 2ml of benedictôs reagent. The mixture was shaken, heated on a water bath for 5minutes.

Observation: A rusty brown precipitate, indicate the presence of reducing sugar.

Result: +

Test for alkaloids

General test: 20ml of 5% sulphuric acid in 50% ethanol was added to about 2g of the powdered material and heated on a boiling water bath for 10minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of:-

Mayerôs reagent

Dragendroffôs reagents

Wegnerôs reagent

Picric acid solution (1%)

The remaining filtrate was placed in 100ml separator funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of dilute sulphuric acid. The extract was tested with a few drops of Mayerôs, Wagnerôs, dragendroffôs reagent and 1% picric acid solution.

Observation: milky precipitate with one drop of Mayerôs reagent

Reddish brown precipitate with one drop of Wagnerôs reagent

Yellow precipitate with one drop of picric acid reagents

Brick red precipitate with one drop of dragendroffôs reagent

Result: +++

Test for glycosides

About 5ml of dilute sulphuric acid were added to about 0.1g of the powder in a test tube and boiled for 15minutes on a water bath, then cooled and neutralized with

20% potassium hydroxide. 10ml of a mixture of equal parts of fehlingôs solutions I and II were added and boiled for 5minutes.

Observation: a more dense brick red precipitate indicates the presence of glycosides.

Result: ++

Test for saponins

About 20ml of water was added to 0.25g of the powder in 100ml beaker and boiled gently on a hot water bath for 2minutes. The mixture was filtered hot and allowed to cool and the filtrate used for the following tests:

Frothing test: About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously.

Observation: A stable foam upon standing indicates the presence of saponins.

Emulsion test: to the frothing solution 2ml of olive oil was added and the content shaken vigorously.

Observation: the formation of emulsion indicates the presence of saponin.

Result: ++

Test for tannins

About 1g of the powdered material was boiled with 50ml of water, filtered and used for the following tests:

Ferric chloride test: To 3ml of the filtrate few drops of ferric chloride were added.

Observation: A greenish black precipitate indicates the presence of tannins.

Lead subacetate test: A few drops of lead subacetate was added to 3ml of the filtrate.

Observation: A cream precipitate appearing would interfere with the presence of tannins.

Result +++

Test for flavonoids

10ml of ethylacetate were added to 0.2g of the powdered leaf material and heated on a water bath for 3minutes. The mixture was cooled, filtered and the filtrate used for the following tests:

Ammonium test: About 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layer were allowed to separate

Observation: The yellow colour in the ammonia layer indicates the presence of flavonoids.

1% Aluminum chloride solution test: Another 4ml portion of the filtrate was shaken with 1ml of 1% aluminum chloride solution. The layers were allowed to separate.

Observation: A yellow colour in the aluminum chloride layer indicates the presence of flavonoids.

Result: ++

Test for resins

Precipitation test: 0.2g of the powdered leaf material was extracted with 15ml of 96% ethanol. The alcoholic extract was then poured into 20ml of distilled water in a beaker.

Observation: A white precipitate indicates the presence of resins.

Result: +

Test for proteins

Millionôs test: To a little portion of the filtrate in a test tube, two drops of millions reagent were added.

Observation: A white precipitate indicates the presence of proteins.

Xanthoproteic reaction: 5ml of the filtrate was heated with few drops of concentrated nitric acid.

Observation: A yellow colour which changes to orange on addition of an alkali indicates the presence of proteins.

Picric acid test: To a little portion of the filtrate was added a few drops of picric acid.

Observation: A yellow precipitate indicates the presence of proteins.

Result: ++

Test for oils

0.1g of the powder was made into paste and pressed between filter paper

Observation: A Translucency of the filter paper indicates the presence of oil.

Result: +

Test for steroids

9ml of ethanol were added to 1g of the powder and refluxing for a few minutes and filtered. The filtrate was concentrated to 2.5ml on boiling water bath, about 5ml of hot water were added. The mixture was allowed to stand for 1hour and the waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer.

Observation: A reddish brown interface shows the presence of steroids.

Result: ++

Test for terpenoids

Another 0.5ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10minutes on a water bath.

Observation: A grey colour indicates the presence of Terpenoids.

Result: +++

Test for Acidic compound

0.1g of powdered leaf was placed in a clear dry test tube and sufficient water added. This was warmed in a hot water bath and then cooled. A piece of water wetted litmus paper was dipped into the filtrate.

Observation: A colour change on the litmus paper indicates trace of acidic compounds.

Result: +

3.12.2 ANALYSIS OF METHANOLIC LEAF EXTRACT OF *OCIMUM T.*

METHOD

150mg of the methanolic extract was reconstituted with 60ml of methanol. The solution was filtered and the 3ml of the filtrate was poured into 10ml test tubes and labeled serially the filtrates was evaporate to dryness using a hot air oven at 65^{o}C . Tests for the secondary metabolites were carried out on the filtrates as follows:

Test for carbohydrate

The residue of the methanol leaf material was boiled with 2ml of water and filtered. To the filtrate, few drops of napthol solution in ethanol (Molischôs reagent) were added. 1ml of concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer.

Observation: A purple interface ring indicates the presence of carbohydrate.

Result: ++

Test for reducing sugars

The residue of the methanol leaf extract was shaken vigorously with 5ml of distilled water and filtered. The filtrate was used in the following tests;

Fehlingôs test: to 1ml portion of the filtrate was added equal volume of fehlingôs solution 1 and 11 and boiled on water bath for few minutes.

Observation: A brick red precipitate, indicate the presence of sugar.

Result: +

Benedictôs test: to 1ml portion of the filtrate was added 2ml of benedictôs reagent. The mixture was shaken, heated on a water bath for 5minutes.

Observation: A rusty brown precipitate, indicate the presence of reducing sugar.

Result: +

Test for alkaloids

General test: 20ml of 5% sulphuric acid in 50% ethanol was added to residue of the leaf material and heated on a boiling water bath for 10minutes, cooled and filtered. 2ml of the filtrate was tested with a few drops of:-

Mayerôs reagent

Dragendoffôs reagent

Wegnerôs reagent

Picric acid solution (1%)

The remaining filtrate was placed in 100ml separator funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of dilute sulphuric acid. The extract was tested with a few drops of Mayerôs, Wagnerôs, dragendroffôs reagent and 1% picric acid solution.

Observation: milky precipitate with one drop of Mayerôs reagent

Reddish brown precipitate with one drop of Wagnerôs reagent

Yellow precipitate with one drop of picric acid reagents

Brick red precipitate with one drop of dragendroffôs reagent

Result: ++

Test for glycosides

About 5ml of dilute sulphuric acid were added to residue in a test tube and boiled for 15minutes in a water bath, then cooled and neutralized with 20% potassium hydroxide. 10ml of a mixture of equal parts of fehlingôs solutions I and II were added and boiled for 5minutes.

Observation: a more dense brick red precipitate indicates the presence of glycosides.

Result: ++

Test for saponins

About 20ml of water was added to the residue of the methanol extract in 100ml beaker and boiled gently on a hot water bath for 2minutes. The mixture was filtered hot and allowed to cool and the filtrate used for the following tests:

Frothing test: About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously.

Observation: stable foam upon standing indicates the presence of saponins.

Emulsion test: to the frothing (foam) solution 2ml of olive oil was added and the content shaken vigorously.

Observation: the formation of emulsion indicates the presence of saponins.

Result: ++

Test for tannins

The residue material was boiled with 50ml of water, filtered and used for the following tests:

Ferric chloride test: To 3ml of the filtrate few drops of ferric chloride were added.

Observation: A greenish black precipitate indicates the presence of tannins.

Lead subacetate test: A few drops of lead subacetate were added to 3ml of the filtrate.

Observation: A cream precipitate appearing would interfere, with the presence of tannins.

Result +++

Test for flavonoids

10ml of ethylacetate were added to the residue of the leaf material and heated in a water bath for 3minutes. The mixture was cooled, filtered and the filtrate used for the following tests:

Ammonium test: About 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layer were allowed to separate

Observation: The yellow colour in the ammonia layer indicates the presence of flavonoids.

1% Aluminum chloride solution test: Another 4ml portion of the filtrate was shaken with 1ml of 1% aluminum chloride solution. The layers were allowed to separate.

Observation: A yellow colour in the aluminum chloride layer indicates the presence of flavonoids.

Result: ++

Test for resins

Precipitation test: the residue of the leaf material was extracted with 15ml of 96% ethanol. The alcoholic extract was then poured into 20ml of distilled water in a beaker.

Observation: A white precipitate indicates the presence of resins.

Result: +

Test for proteins

Millionôs test: To a little portion of the filtrate in a test tube, two drops of millions reagent were added.

Observation: A white precipitate indicates the presence of proteins.

Xanthoproteic reaction: 5ml of the filtrate was heated with few drops of concentrated nitric acid.

Observation: A yellow colour which changes to orange on addition of an alkali indicates the presence of proteins.

Picric acid test: To a little portion of the filtrate was added a few drops of picric acid.

Observation: A yellow precipitate indicates the presence of proteins.

Result: ++

Test for oils

0.1g of the methanol extract was pressed between filter paper

Observation: A Translucency of the filter paper indicates the presence of oil.

Result: +

Test for steroids

9ml of ethanol were added to the residue of the methanol and refluxing for a few minutes and filtered. The filtrate was concentrated to 2.5ml on boiling water bath; about 5ml of hot water were added. The mixture was allowed to stand for 1hour and filtered. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer.

Observation: A reddish brown interface shows the presence of steroids.

Result: ++

Test for terpenoids

Another 0.5ml of the chloroform extract was evaporated to dryness and heated with 3ml of concentrated sulphuric acid for 5minutes in a water bath.

Observation: A grey colour indicates the presence of Terpenoids.

Result: +++

Test for Acidic compound

The residue of the leaf extract was placed in a clear dry test tube and sufficient water added. This was warmed in a hot water bath and then cooled. A piece of water wetted litmus paper was dipped into the filtrate.

Observation: A colour change on the litmus paper indicates trace of acidic compounds.

Result: +

3.12.3 ANALYSIS OF AQUEOUS LEAF EXTRACT OF *OCIMUM T.*

METHOD

100mg of the aqueous extract was reconstituted with 50ml of water. The solution was filtered and the 3ml of the filtrate was poured into 10ml test tubes and labeled serially. Tests for the secondary metabolites were carried out on the filtrates as follows:

Test for carbohydrate

To 2ml of aqueous filtrate, few drops of napthol solution in ethanol (Molischôs reagent) were added. Concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer.

Observation: A purple interface ring indicates the presence of carbohydrate.

Result: ++

Test for reducing sugars

3ml of the aqueous filtrate was shaken vigorously with 1ml of distilled water

Fehlingôs test: to 1ml portion of the filtrate was added equal volume of fehlingôs solution 1 and 11 and boiled on water bath for few minutes.

Observation: A brick red precipitate, indicate the presence of sugar.

Result: +

Benedictôs test: to 1ml portion of the filtrate was added 2ml of benedictôs reagent. The mixture was shaken, heated on a water bath for 5minutes.

Observation: A rusty brown precipitate, indicate the presence of reducing sugar.

Result: +

Test for alkaloids

2ml of 5% sulphuric acid in 50% ethanol was added to 3ml of aqueous extract in a boiling water bath, allowed to cool and was tested with a few drops of:-

Mayerôs reagent

Dragendoffôs reagent

Wegnerôs reagent

Picric acid solution (1%)

The remaining mixture was placed in 100ml separator funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of dilute sulphuric acid. The extract was tested with a few drops of Mayerôs, Wagnerôs, dragendoffôs reagent and 1% picric acid solution.

Observation: milky precipitate with one drop of Mayerôs reagent

Reddish brown precipitate with one drop of Wagnerôs reagent

Yellow precipitate with one drop of picric acid reagents

Brick red precipitate with one drop of dragendroffôs reagent

Result: +

Test for glycosides

2ml of dilute sulphuric acid were added to 2ml of aqueous filtrate in a test tube and boiled for 5minutes in a water bath, then cooled and neutralized with 20% potassium hydroxide. 3ml of a mixture of equal parts of fehlingôs solutions I and II were added and boiled for 2minutes.

Observation: a more dense brick red precipitate indicates the presence of glycosides.

Result: ++

Test for saponins

5ml of aqueous extract was poured in 10ml test tube and boiled gently on a hot water bath for 2minutes. The solution was allowed to cool and used for the following tests:

Frothing test: 3ml of the aqueous was diluted with 3ml of water and shaken vigorously.

Observation: Formation of stable foam upon standing indicates the presence of saponins.

Emulsion test: to the frothing solution 2ml of olive oil was added and the content shaken vigorously.

Observation: the formation of emulsion indicates the presence of saponin.

Result: ++

Test for tannins

Ferric chloride test: To 3ml of the aqueous filtrate, few drops of ferric chloride was added.

Observation: A greenish black precipitate indicates the presence of tannins.

Lead subacetate test: A few drops of lead subacetate was added to 3ml of the aqueous filtrate.

Observation: A cream precipitate appearing would interfere, with the presence of tannins.

Result +++

Test for flavonoids

10ml of ethylacetate were added to aqueous leaf material and heated on a water bath for 3minutes. The mixture was cooled, and used for the following tests:

Ammonium test: 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. The layer were allowed to separate

The yellow colour in the ammonia layer indicates the presence of flavonoids.

Observation: No colour change observed.

(b) 1% Aluminum chloride solution test: Another 4ml portion of the filtrate was shaken with 1ml of 1% aluminum chloride solution. The layers were allowed to separate. A yellow colour in the aluminum chloride layer indicates the presence of flavonoids.

Observation: Absent of yellow colour

Result: Negative (-)

Test for resins

Precipitation test: the aqueous leaf material was extracted with 15ml of 96% ethanol. The alcoholic extract was then poured into 20ml of distilled water in a beaker. A white precipitate indicates the presence of resins.

Observation: No white precipitate observed

Result: Negative (-)

Test for proteins

Millionôs test: To 1ml portion of the filtrate in a test tube, two drops of millions reagent were added, mixed and allowed to stand for few minutes.

Observation: A white precipitate indicates the presence of proteins.

Xanthoproteic reaction: 5ml of the filtrate was heated with few drops of concentrated nitric acid.

Observation: A yellow colour which changes to orange on addition of an alkali indicates the presence of proteins.

Picric acid test: To 1ml portion of the filtrate was added a few drops of picric acid.

Observation: A yellow precipitate indicates the presence of proteins.

Result: ++

Test for oils

The aqueous extract was made into paste and pressed between filter paper

Observation: A Translucency of the filter paper indicates the presence of oil.

Result: +

Test for steroids

9ml of ethanol were added to 5ml of aqueous extract and refluxing for a few minutes and filtered. The filtrate was concentrated to 2.5ml on boiling water bath 5ml of hot water were added. The mixture was allowed to stand for 1hour and the

waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating funnel. To 0.5ml of the chloroform extract in a test tube was carefully added 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface shows the presence of steroids.

Observation: Absence of reddish brown interface

Result: Negative (-)

Test for terpenoids

1ml of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 2minutes on a water bath. A grey colour indicates the presence of Terpenoids.

Observation: No grey colour observed

Result: Negative (-)

Test for Acidic compound

3ml of the leaf extract was placed in a clear dry test tube and sufficient water added. This was warmed in a hot water bath and then cooled. A piece of water wetted litmus paper was dipped into the filtrate. A colour change on the litmus paper indicates trace of acidic compounds.

Observation: No colour change observed

Result: Negative (-)

CHAPTER FOUR

RESULTS:

The result of acute toxicity studies revealed that the methanol and aqueous extract of Ocimum tenuiflorum leaf had an oral LD_{50} greater than 5000mg/kg body weight in albino rats.

The qualitative phytochemical analysis of Ocimum tenuiflorum leaf extract showed the presence of carbohydrates, reducing sugar, alkaloids, glycosides, Saponins, tannins, flavonoids, resins, proteins, oil, steroids, terpenoids, and acidic compound (Table 4). Table 1 showed that immunosupression was achieved by intra peritoneal administration of cyclophosphamide in the albino rats.

Effect of *Ocimum tenuiflorum* leaf extract on Hb, pcv, total wbc count and leucocyte differential count in albino rats.

Group B (CP control) of day 15 showed significantly decreased value in Hb; 5.8 ± 0.30g/dl, PCV; 17.58 ± 0.74%, the leucocytes were very few Neut; 0.75 ± 1.5%, lymph; 3.5 ± 4.73%, Mono 0 ± 00%, and Eos; 0 ± 00% compared to normal control group A: Hb; 13.65 ± 0.47g/dl, PCV; 41.15 ± 1.35%, Neut; 22.25 ± 6.55%, Lym; 73.75 ± 6.75%, Mono; 2.5 ± 1.73%, Eos; 1.75 ± 1.26% ($P<0.001$).

On the same day 15 the mean HB concentration revealed significantly increased value in groups (C) 11.27 ± 0.85g/dl, (D) 12 ± 0.30g/dl, (E) 11.67±0.38g/dl, (F) 12.45 ± 0.31g/dl, compared to CP control group (B) 5.875 ± 0.30g/dl, ($p<0.05$).

PCV of day 15 revealed significantly increased value in groups (C) 33.47±2.17%, (D)36.53±0.50%, (E) 35.5± 1.67%, (F) 37.35±1.12%, compared to CP control group(B) 17.58±0.74%, ($p<0.01$).

Neutrophils were significantly elevated in (C) 63.33±10.60%, (D) 54.33±15.70%, (E) 38.33±6.66%, (F) 77.75±11.00%, compared to CP control group (B) 0.75±1.50% ($p<0.001$).

Lymphocyte count was significantly raised in groups (C) 30±14.73%, (D) 42 ± 14.18%, (E) 54.67±8.08%, (F) 21.75±11.21%, when compared to (B) 3.5±4.73%, ($p<0.001$).

Monocyte count revealed increased value in (C) 2.333±1.53%, (D) 3±1.00%, compared to (B) 0± 0.00%, ($p<0.003$).

Eosinophil count were significantly increased in groups (C) 2.67±3.06%, (D) 0.67±1.15%, (E) 2±2.00%, when compared to CP control group (B) 0±0.00%,($p<0.01$).

Total WBC significantly increased in groups (C) 23.87 ± 24.16 $x10^{3/\mu l}$, (D) 12.07± 2.8 $x10^{3/\mu l}$, (E) 14.97 ± 4.24 $x10^{3/\mu l}$, and (F) 14.3 ± 7.40 $x10^{3/\mu l}$ compared CP control group (B) 0.63 ± 0.21 $x10^{3/\mu l}$, ($P<0.001$).

On Day 22 The mean HB concentration revealed significantly decreased value in group (B) 5.633±0.85g/dl CP control, compared to groups (C) 11.65±0.21g/dl, (D) 13.15±0.49g/dl, (E) 12.25±0.07g/dl, (F) 13.8g/dl, ($p<0.05$).

The packed cell volume were significantly increased in groups (C) 35.2±0.85%, (D) 39.85±1.34%, (E) 36.95±0.21%, (F) 41.9%, compared to CP control group (B) 17.43±2.23%, ($p<0.01$).

Neutrophil count was significantly elevated in groups (D) 60±14.40%, and (F) 52% compared to (B) 38±4.24% ($p<0.001$).

Lymphocyte count were significantly increased in groups (C) 55.5±12.02%, (E) 60±7.07%, compared to CP control group (B) 54.64±7.57%, ($p<0.05$).

Monocyte count was also significantly raised in groups (C) 2±0.00 % (D) 1.5±2.12% and (F)2%, compared to CP control group (B) 1±1.00% ($p<0.05$).

Eosinophil count revealed significantly increased value in groups (C) 5±7.07%, and (E) 4±1.41% compared to (B) 1.33±1.53%, ($p<0.001$).

Generally there was no statistical significant difference between groups G & H (none induced) on days 15 and 22 when compared with the normal control group A.

Groups G&H of day 15, HB; 12.98 ± 0.67g/dl, 12.2 ± 0.40g/dl, PCV; 40.27± 3.31%, and 37.2±1.51%, Neut; 26±8.72%, and 20±2.00%, Lym; 73.67±8.50%, and 79±1.73%, when compared with the normal control group A, HB; 13.65 ± 0.47g/dl, PCV; 41.15 ± 1.35%, Neut; 22.25 ± 6.55%, Lym; 73.75 ± 6.75%, ($P>0.05$).

Groups G&H of day 22, HB; 13.43±1.33g/dl, and 13.93±0.23g/dl, PCV; 40.73±4.28% and 41.63±0.72%, Neut; 35±16.52% and 19.67±9.69%, lym; 64±16.52%, and 78±7.21% when compared with the normal control group A, HB; 14.53±0.87%, PCV; 43.93±2.77%, Neut; 18.75±3.77%, Lym; 79.25±4.65%, $P>0.05$

No statistical significant changes was observed in total white cell count following the oral administration of the leaf extract on day 22, ($P<0.399$)**.**

TABLE 4.1 DAY 15 The Effect of Ocimum tenuiflorum leaf extract on Haematological parameters of immunosuppressed albino rats.

TEST	GP A normal Control	GP B CP control	GP C CP +500mg/kg AE	GP D CP + 1000mg/kg AE	GP E CP +500mg/kg ME	GP F CP + 1000mg/kg	GP G + 500mg/kg AE	GP H +1000 mg/kg ME
HB g/dl	13.65±0.5	5.88±0.3	11.27±0.9	12±0.3*	11.67±0.4	12.45±0.3*	12.98±0.7	12.2±0.4
PCV%	41.15±1.4	17.58±0.7	33.47±2.2*	36.53±0.5**	35.5±1.7*	37.35±1.1**	40.27±3.3	37.2±1.5
Neut%	22.25±6.6	0.75±1.5	63.33±10.6***	54.33±15.7***	38.33±6.7*	77.75±11.0***	26±8.7	20±2.0
Lymp%	73.75±6.8	3.5±4.7	30±14.7*	42±14.2**	54.67±0.1***	21.75±11.2*	73.67±8.5	79±1.7
Eos%	1.75±1.3	0±0.0	2.67±3.1*	0.67±1.2	2±2.0*	0.25±0.5	0.0±0.0	0.33±0.6
Mono%	2.5±1.7	0±0.0	2.33±1.5**	3±1.0**	0±0.0	0.25±0.5	0.33±0.6	0.67±1.2
Wbc $x10^{3/\mu l}$	11.33±6.9	0.63±0.2	23.87±24.**2	12.07±2.8	14.97±4.2	14.3±7.4	13.93±5.2	10.13±3.8

CP = Group

ME = Methanol Extract

AE = Aqueous Extract

CP = Cyclophosphamide induced

P = Probability value

P<0.05 = Significant

*P<0.5, **P<0.01

***P<0.001

TABLE 4.2 DAY 22 The Effect of Ocimum tenuiflorum leaf extract on Haematological parameters of immunosuppressed albino rats.

TEST	GP A normal control	GP B CP control	GP C CP+500 mg/kg AE	GP D CP +1000mg/ kg AE	GP E CP+ 500mg/k g ME	GP F CP+100 0mg/kg	GP G + 500mg/k g AE	GP H + 1000m g/kg ME
HB g/dl	14.53±0 .87	5.63±0. 85	11.65±0.2 1	13.15±0.4 9	12.25±0. 07	13.8	13.43±1. 33	13.93± 0.23
PCV%	43.93±2 .77	17.43±2 .23	35.2±0.85	39.85±1.3 4	36.95±0. 21	41.9	40.73±4. 28	41.63± 0.72
Neut %	18.75±3 .77	38±4.24	37.5±4.95	60±14.40* *	35±7.07	52**	35±16.5 2	19.67± 9.69
Lymp%	79.25±4 .65	54.67±7 .57	55.5±12.0 2*	37.5±10.6 1	60±7.07 **	46	64±16.5 2	78±7.2 1
Mono%	1.5±1.2 9	1±1.00	2±0.00	1.5±2.12	1±1.41	2	0.33±0.5 8	0.67±1 .15
Eos%	0.5±0.5 8	1.33±1. 53	5±7.07**	1±1.41	4±1.41* *	0	0.67±0.5 8	1.67±1 .53
Wbc x$10^{3/\mu l}$	7.43±2. 51	18.93±1 2.98	10.95±5.7 3	14.3±4.38	19.5±14. 45	8.6	12.3±4.7 3	12.5±3 .41

GP = Group

CP = Cyclophosphamide induced

AE = Aqueous Extract

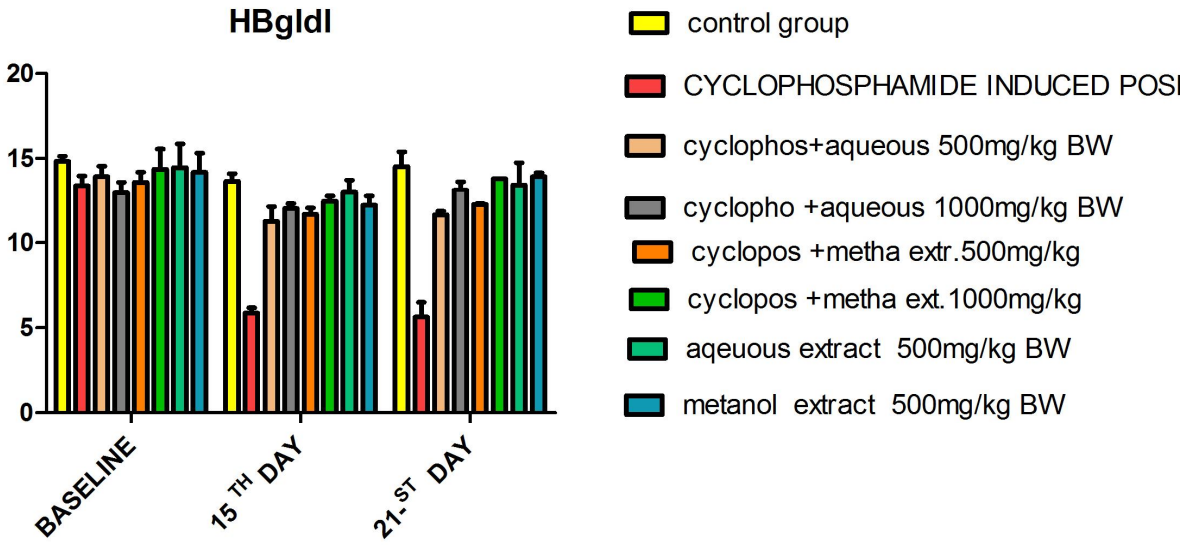
ME = Methanol Extract

P = probability value

P<0.05 = significant

*P<0.05, **P<0.01

***P<0.001


HBgldl
20
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5
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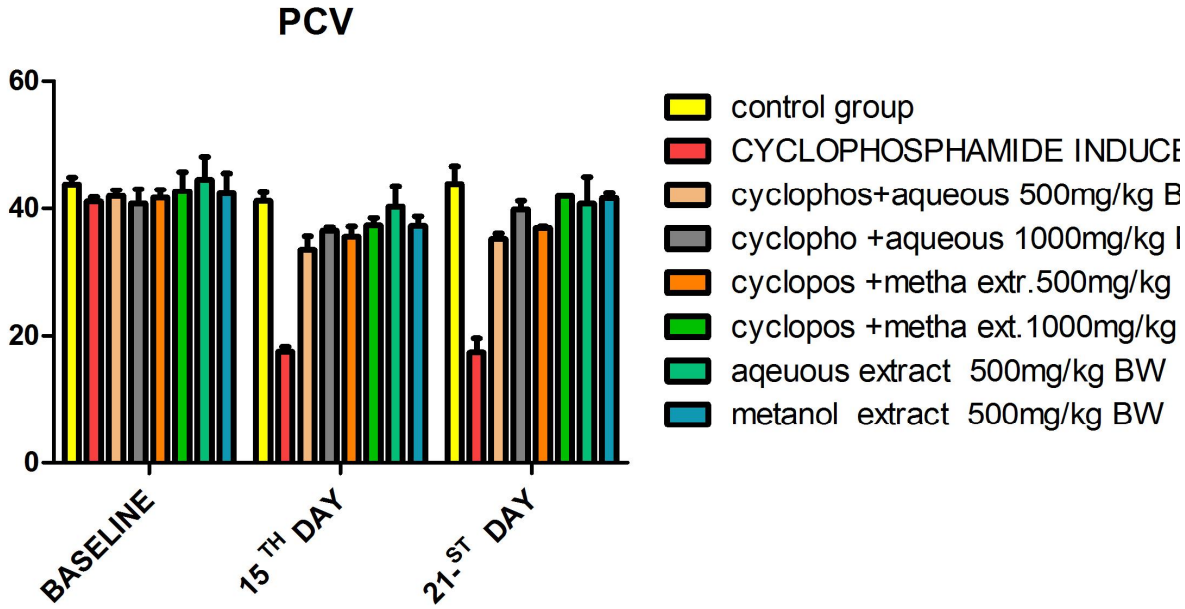
BASELINE
15 TH DAY
21-ST DAY
control group
CYCLOPHOSPHAMIDE INDUCED POSI
cyclophos+aqueous 500mg/kg BW
cyclopho +aqueous 1000mg/kg BW
cyclopos +metha extr.500mg/kg
cyclopos +metha ext.1000mg/kg
aqeuous extract 500mg/kg BW
metanol extract 500mg/kg BW


PCV
60
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control group
CYCLOPHOSPHAMIDE INDUCE
cyclophos+aqueous 500mg/kg B
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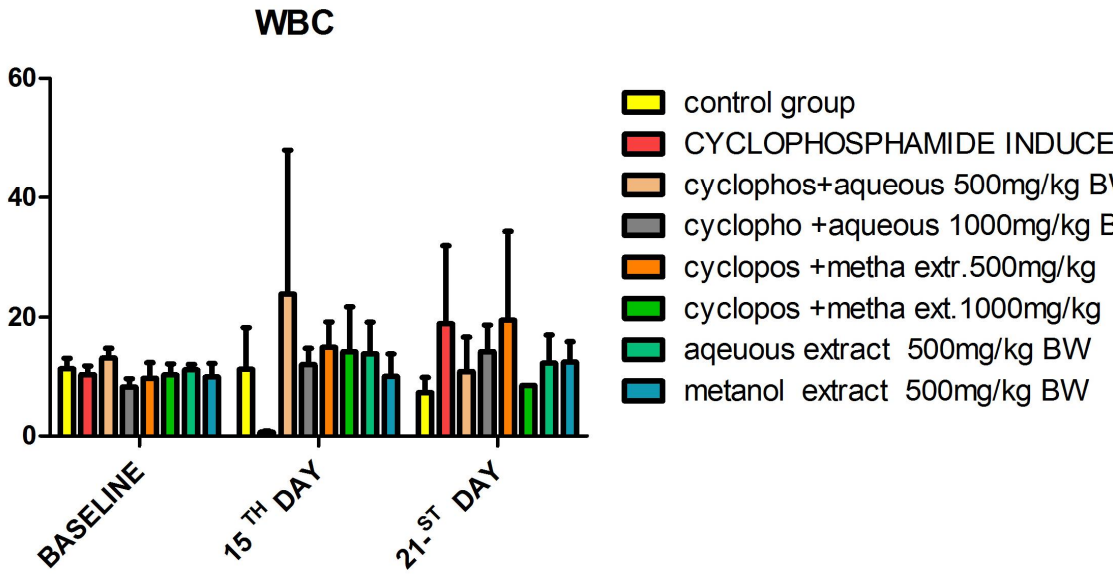
cyclopos +metha ext.1000mg/kg
aqeuous extract 500mg/kg BW
metanol extract 500mg/kg BW


WBC
60
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BASELINE
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control group
CYCLOPHOSPHAMIDE INDUCE
cyclophos+aqueous 500mg/kg B
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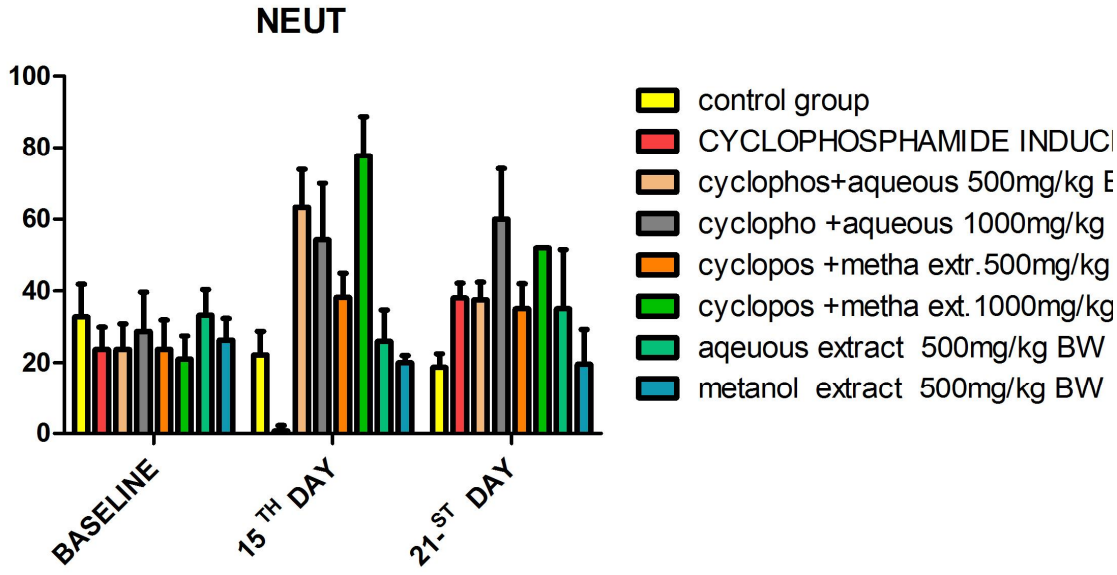
cyclopos +metha extr.500mg/kg
cyclopos +metha ext.1000mg/kg
aqeuous extract 500mg/kg BW
metanol extract 500mg/kg BW


NEUT
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control group
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cyclopos +metha extr.500mg/kg
cyclopos +metha ext.1000mg/kg
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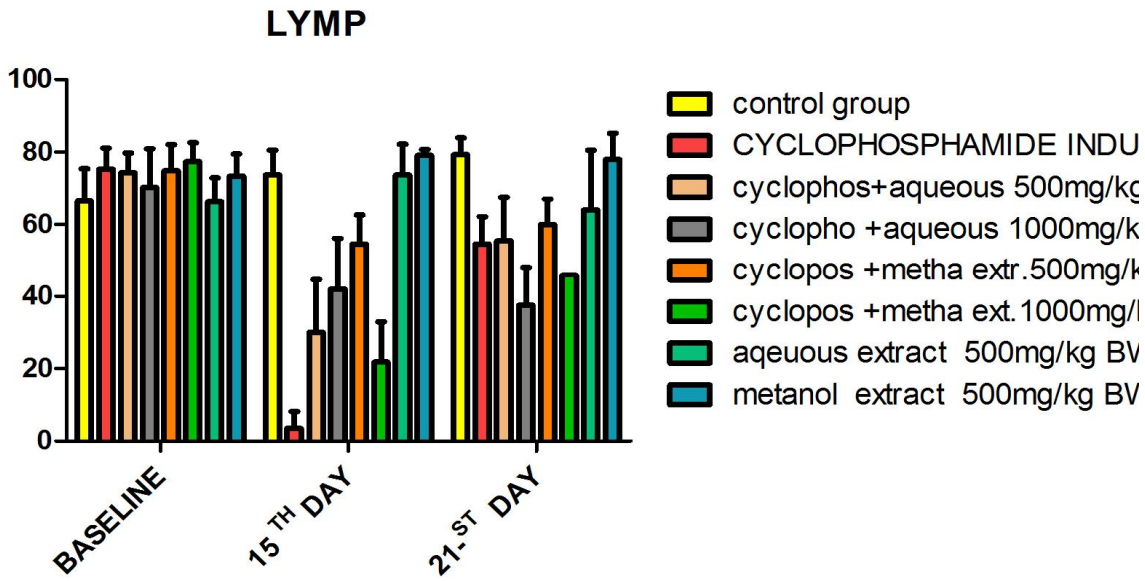
metanol extract 500mg/kg BW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BASELINE
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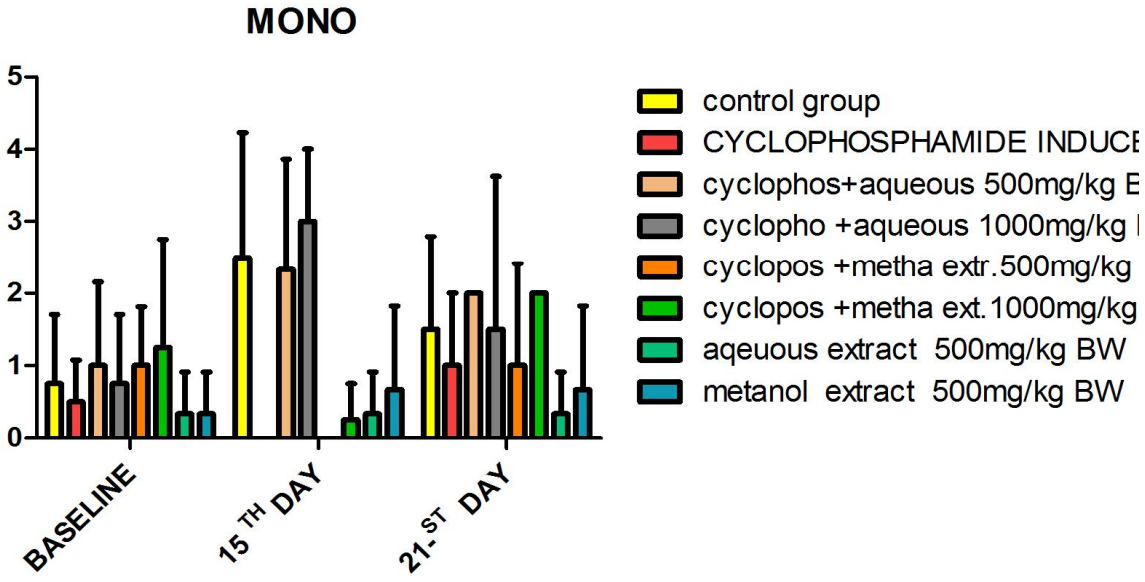
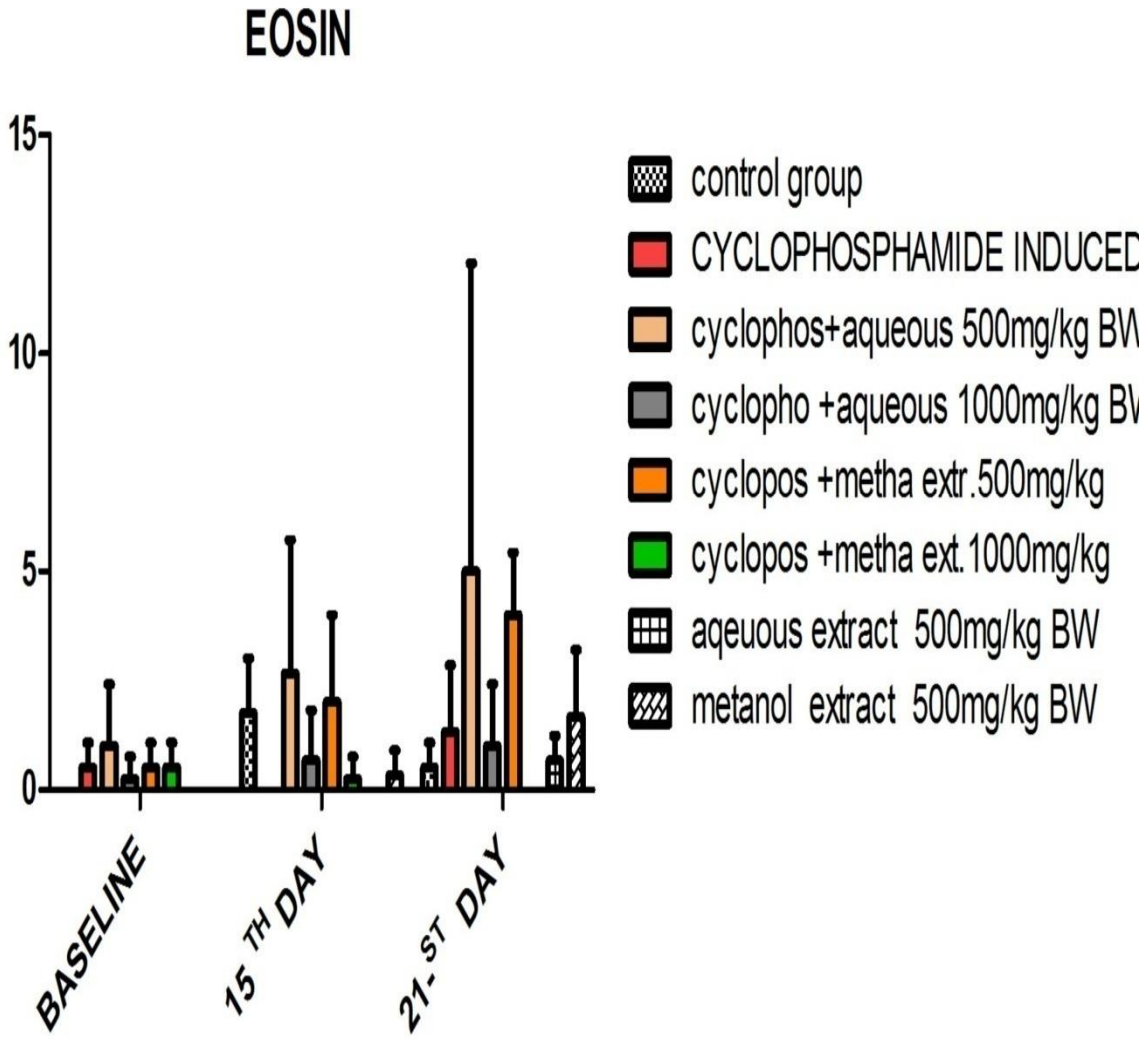
21- ST DAY
control group
CYCLOPHOSPHAMIDE INDU
cyclophos+aqueous 500mg/kg
cyclopho +aqueous 1000mg/k
cyclopos +metha extr.500mg/k
cyclopos +metha ext.1000mg/
aqeuous extract 500mg/kg BV
metanol extract 500mg/kg BV


MONO
5
4
3
2
1
0
BASELINE
15 TH DAY
21- ST DAY
control group
CYCLOPHOSPHAMIDE INDUCE
cyclophos+aqueous 500mg/kg E
cyclopho +aqueous 1000mg/kg
cyclopos +metha extr.500mg/kg
cyclopos +metha ext.1000mg/kg
aqeuous extract 500mg/kg BW
metanol extract 500mg/kg BW


EOSIN
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BASELINE
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control group
CYCLOPHOSPHAMIDE INDUCED
cyclophos+aqueous 500mg/kg BW
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cyclopos +metha extr.500mg/kg
cyclopos +metha ext.1000mg/kg
aqeuous extract 500mg/kg BW
metanol extract 500mg/kg BW

TABLE 4.3

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *OCIMUM TENUIFLORUM* LEAF EXTRACT.

Phytochemicals	Crude powder results	Methanol Extract results	Aqueous Extract results
Carbohydrates	+++	++	++
Reducing sugar	+	+	+
Alkaloids	+++	++	+
Glycosides	++	++	++
Saponins	++	++	++
Tannins	+++	+++	+++
Flavonoids	++	+	–
Resins	+	+	–
Proteins	++	++	++
Oils	+	++	+
Steroids	++	++	–
Terpenoids	+++	+++	–
Acidic compound	+	+	–

– Phytochemical absent

+ Phytochemical low in concentration

++ Moderate concentration

+++ High concentration

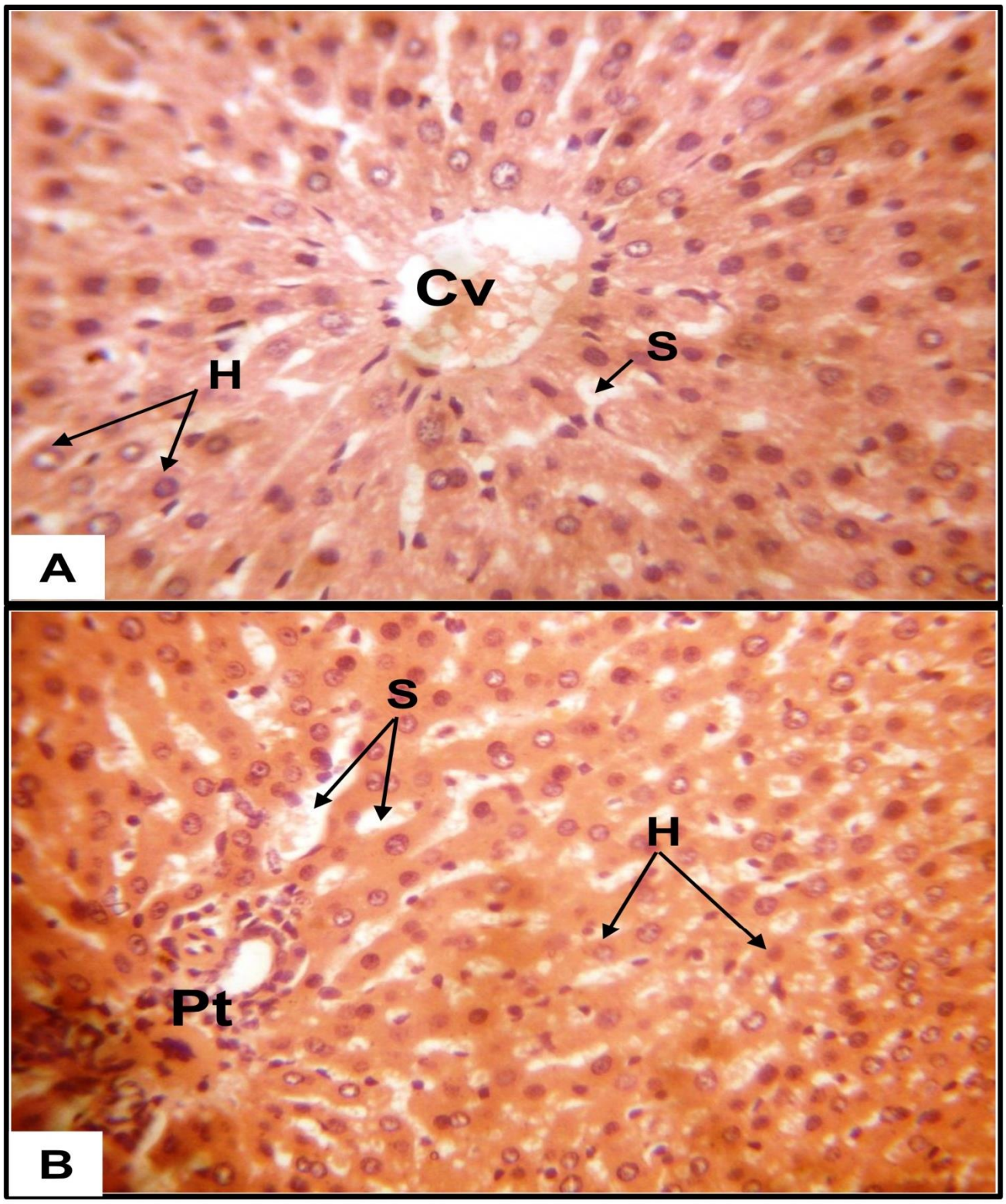

PLATE 1: Liver section photomicrographs from rat in normal Control group showing normal features of the hepatic tissue. The central vein [Cv], portal tract [Pt], hepatocytes [h] and sinusoids [s] shown, are normal.
[STAIN: H& E] [Mag: A – x400; B – x400]

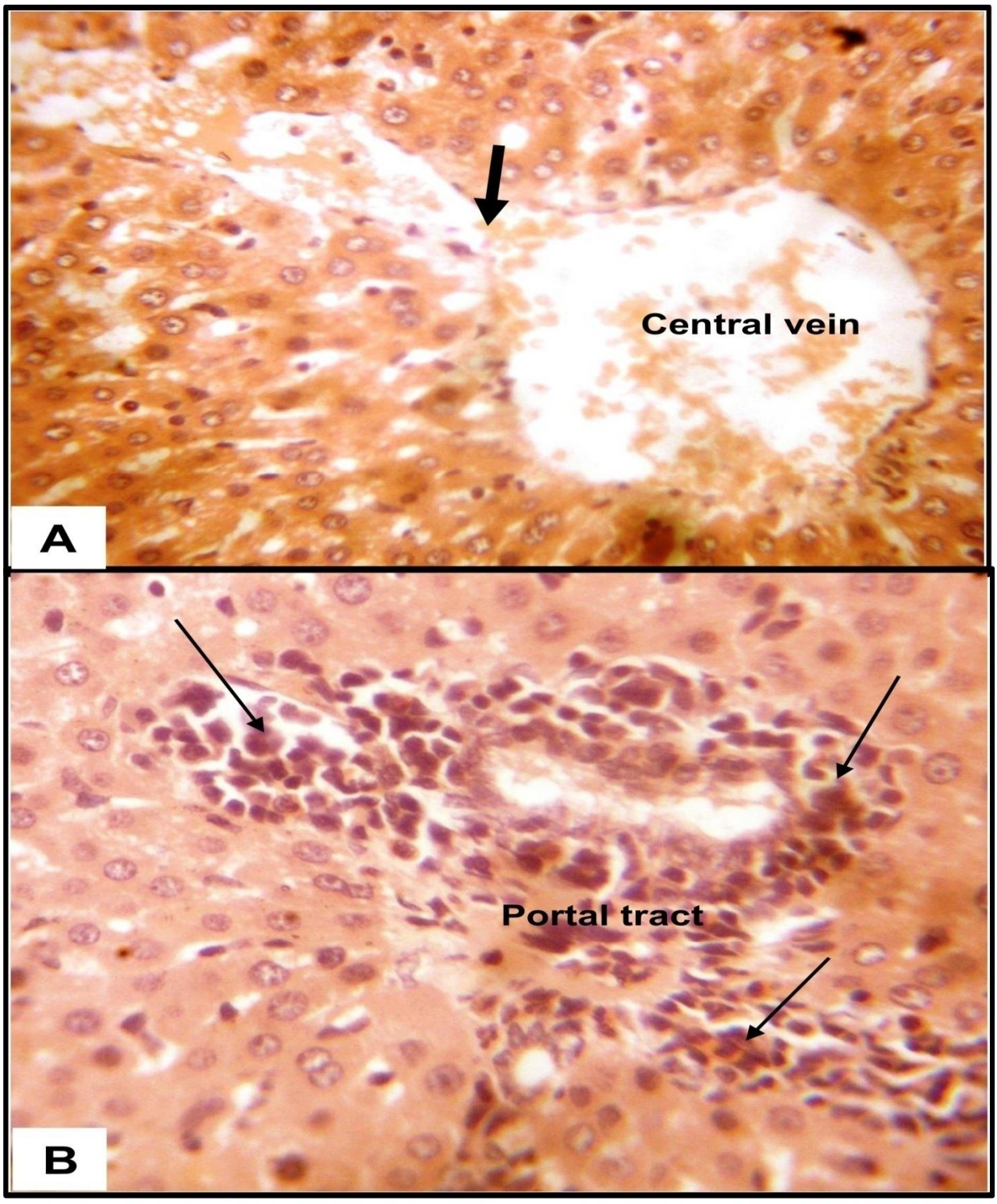

PLATE 2: Photomicrographs from Liver section of rat in Cyclophosphamide [CYCLO] control group showing ruptured central vein [thick arrow] and inflammatory cellular infiltration at periportal region [thin arrows].
[STAIN: H& E] [Mag: A ï x400; B ï x400]

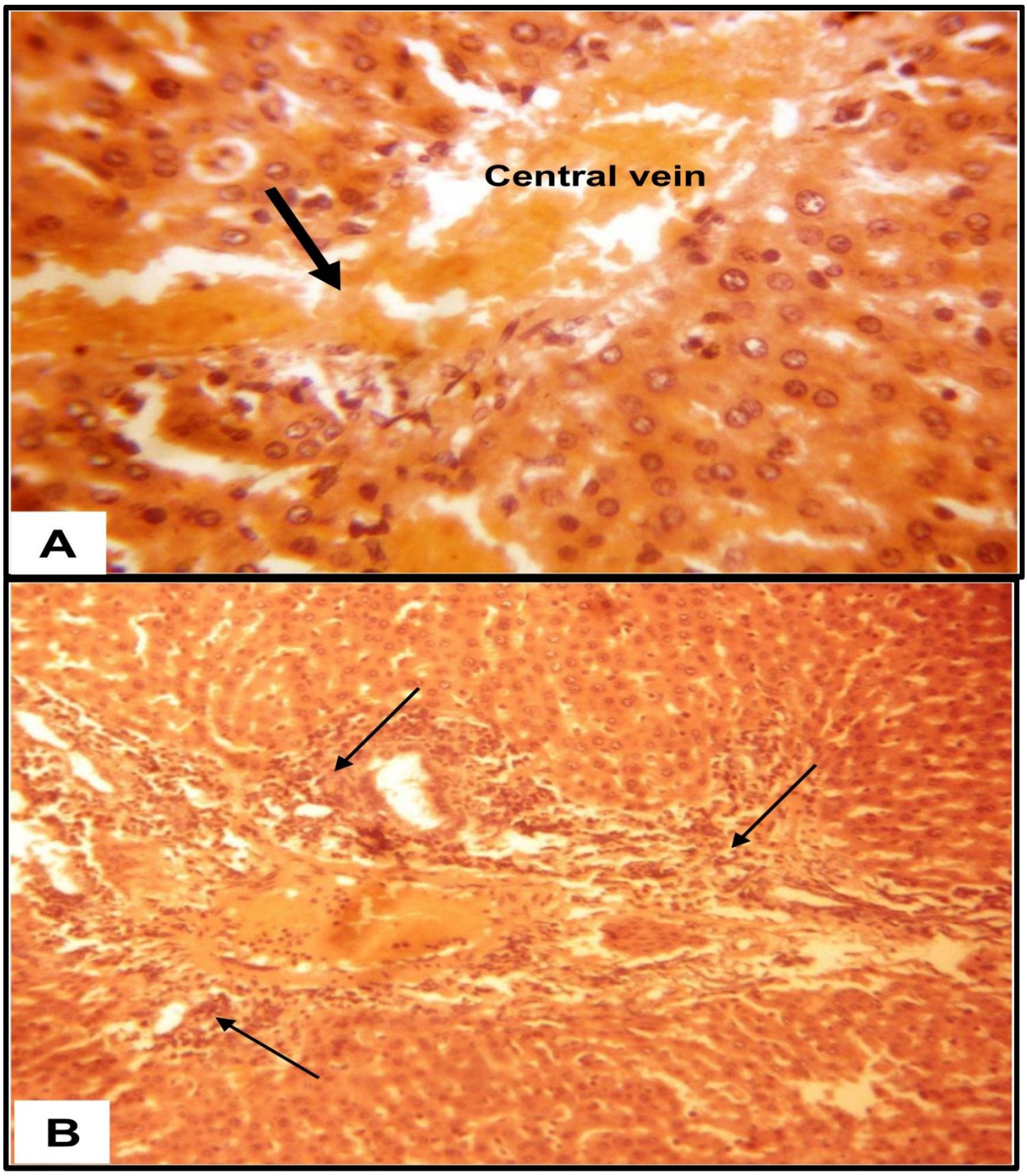

PLATE 3: Photomicrographs from Liver section of rat in Group C treated with CYCLO and 500mg/kgb.wt. of AE showing rupture of the central vein [thick arrow]. Marked inflammatory cellular infiltration and degeneration of surrounding hepatocytes [thin arrows] are evident.
[STAIN: H& E] [Mag: A – x400; B – x100]

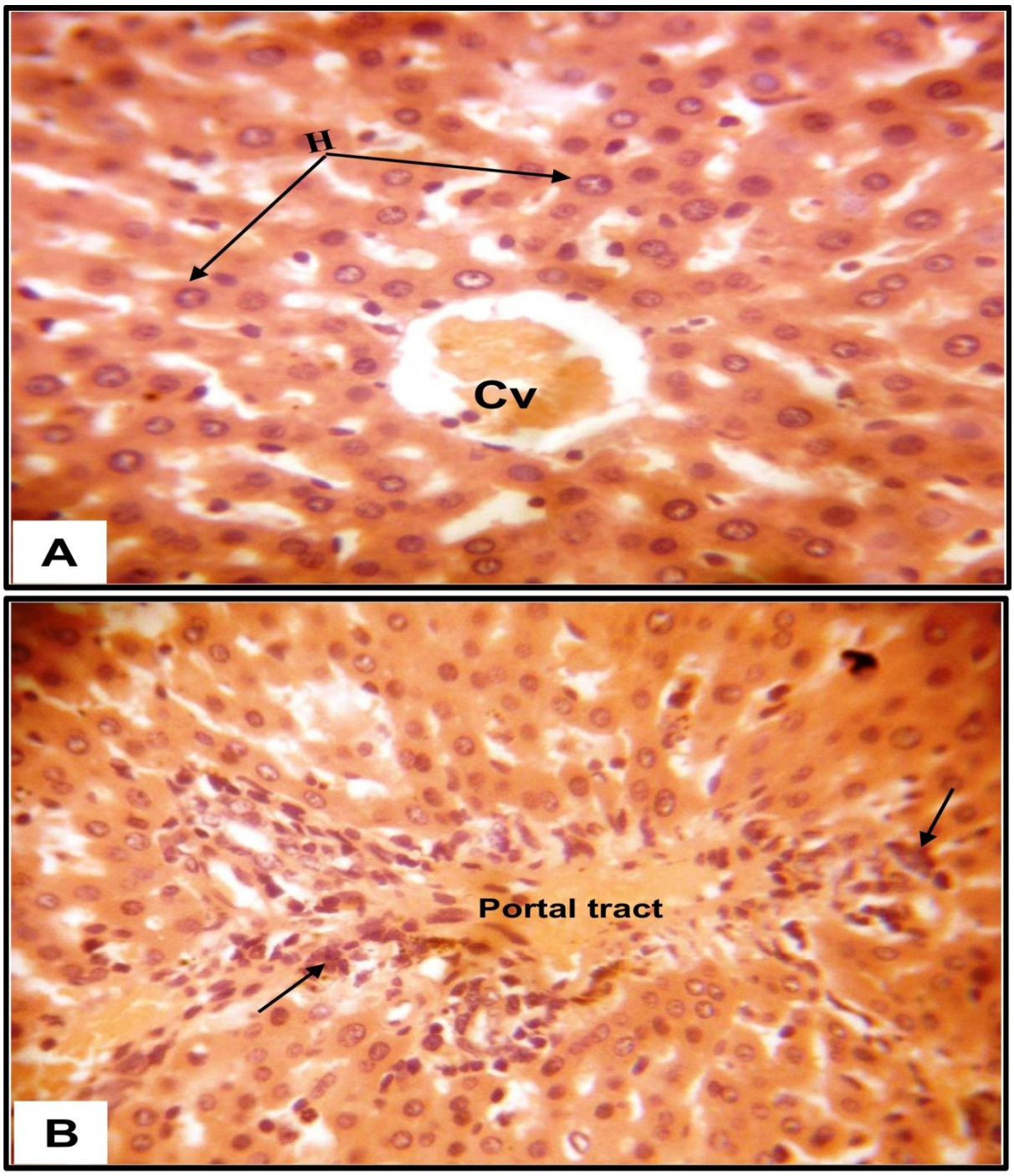

PLATE 4: Photomicrographs from Liver section of rat in Group D treated with CYCLO and 1000mg/kg b.wt. of AE showing intact central vein [Cv] and hepatocytes [H] at pericentral regions. However, mild cellular infiltration is evident within the portal tract [arrow]

[STAIN: H& E] [Mag: A – x400; B – x400]

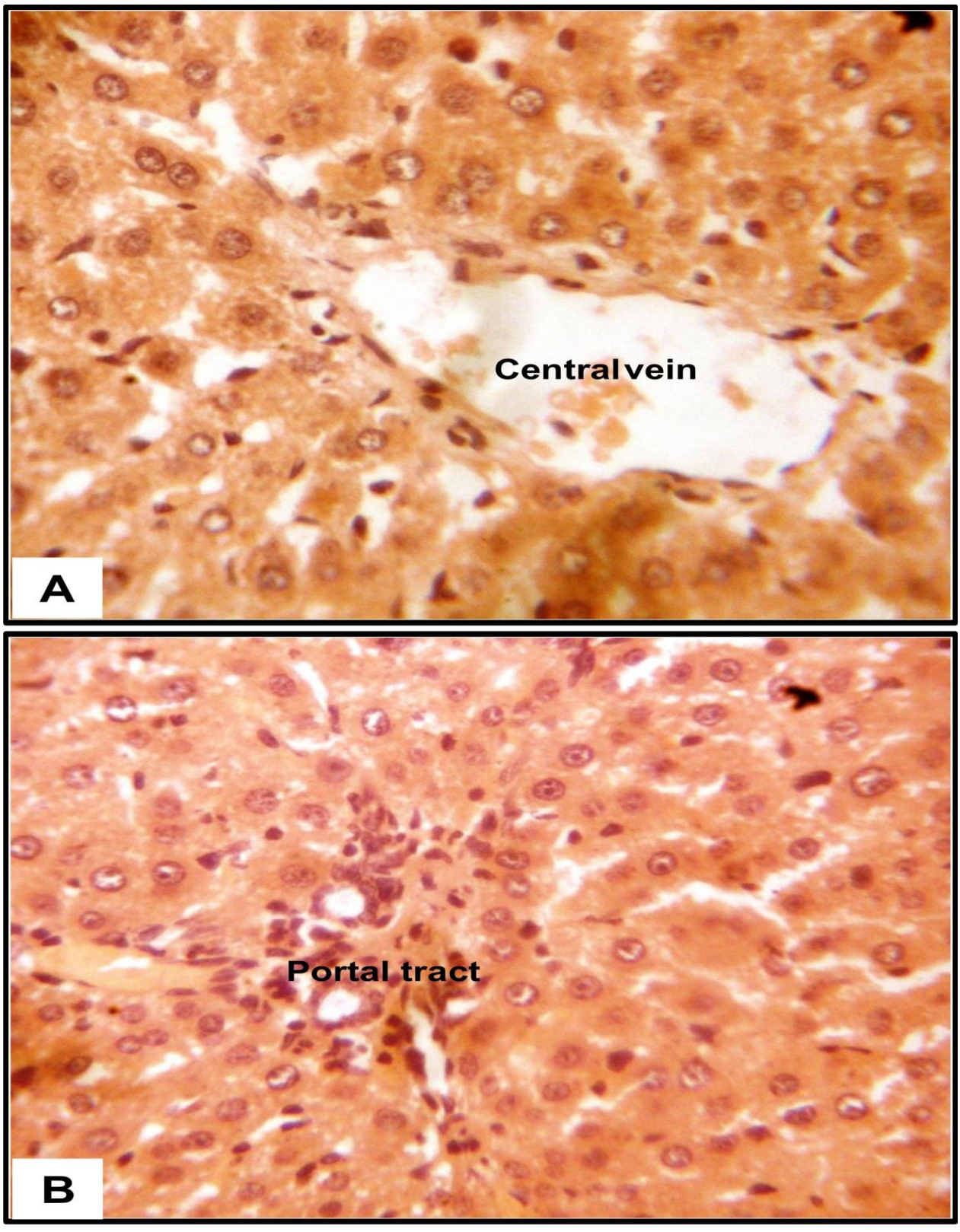

PLATE 5: Photomicrographs from Liver section of rat in Group E treated with CYCLO and 500mg/kg b.wt. of ME showing normal histoarchitecture of the hepatic tissue with no obvious histopathological alteration.
[STAIN: H& E] [Mag: A – x400; B – x400]

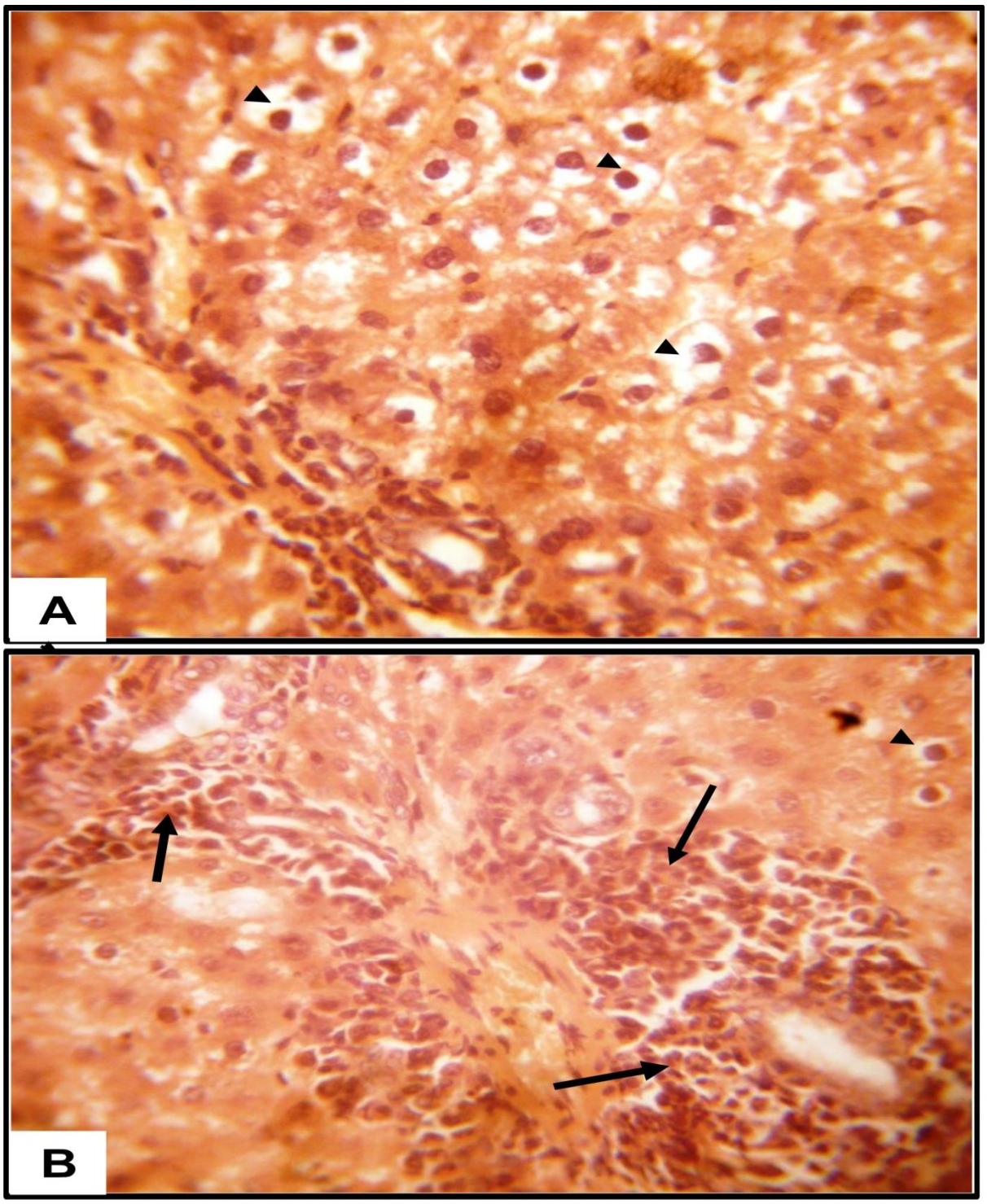

PLATE 6: Photomicrographs from Liver section of rat in Group F treated with CYCLO and with 1000mg/kg b.wt. of ME showing marked cellular infiltration within necrotic hepatocytes at periportal region [arrows]. Degenerating hepatocytes with perinuclear halo are also evident [arrow heads].
[STAIN: H& E] [Mag: A – x400; B – x400]

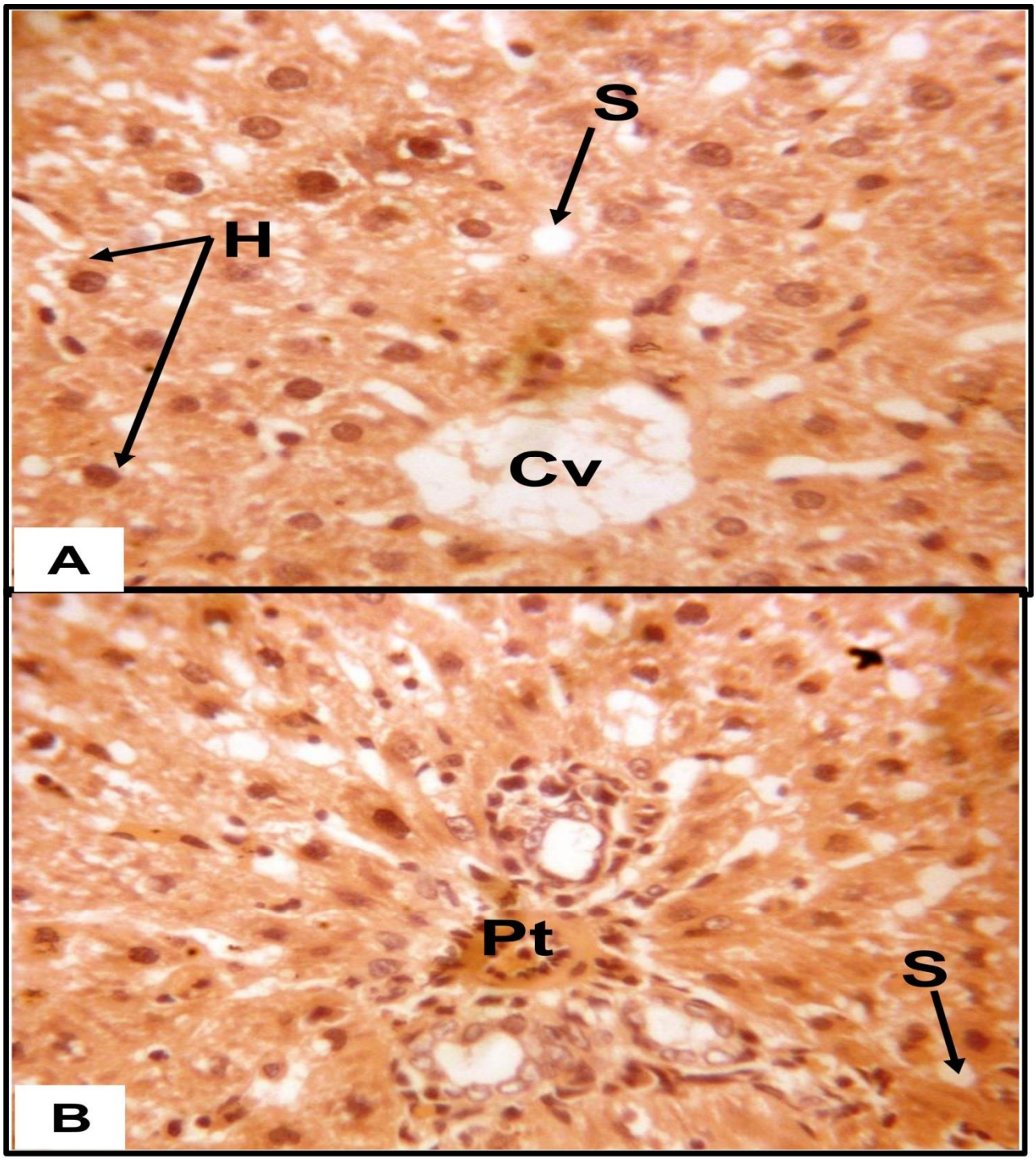

PLATE 7: Photomicrographs from Liver section of rat in Group G treated with 500 mg/kg b.wt. of AE showing no obvious histopathological changes. The central vein [Cv], portal tract [Pt], hepatocytes [H] and sinusoids [s] appear intact.

[STAIN: H& E] [Mag: A – x400; B – x400]

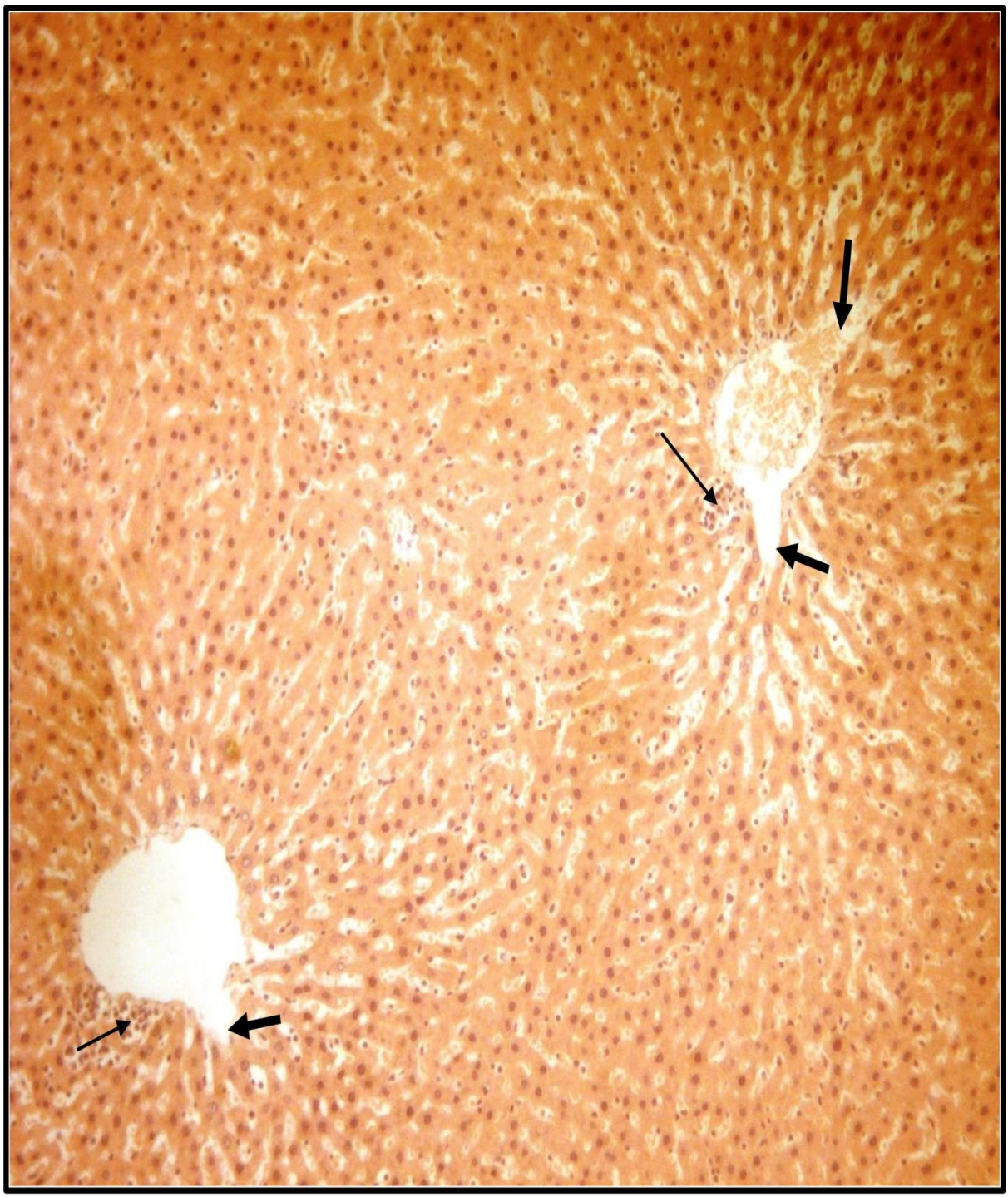

PLATE 8: Photomicrograph from Liver section of rat in Group H treated with 1000 mg/kg b.wt. of ME showing mildly ruptured central vein [thick arrows], mild cellular infiltration [thin arrows] and widened sinusoids at pericentral regions. However, hepatocytes within the lobules appear intact.

[STAIN: H& E] [Mag: x100]

regions. However, hepatocytes within the lobules appear intact.
[STAIN: H& E] [Mag: x100]

PLATE 4: Photomicrographs from Liver section of rat in Group D treated with CYCLO and 1000mg/kg b.wt. of AE showing mild cellular infiltration is evident within the portal tract [arrow]
[STAIN: H& E] [Mag: B x 400]

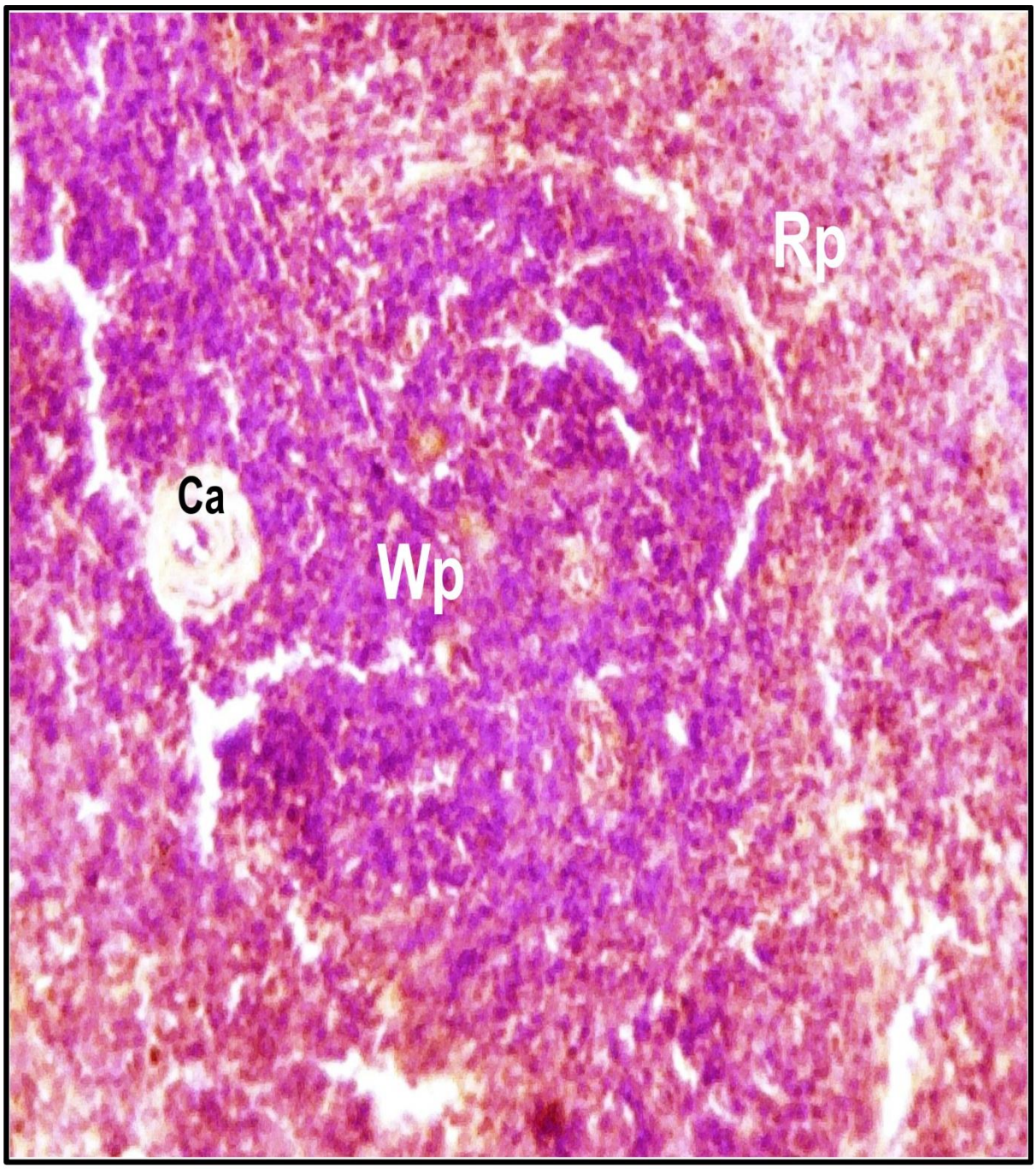

PLATE 9: Photomicrograph from Spleen section of Control rat [Group A] showing normal central artery, white pulp [Wp] and red pulp [Rp].
[STAIN: H& E] [Mag: x400]

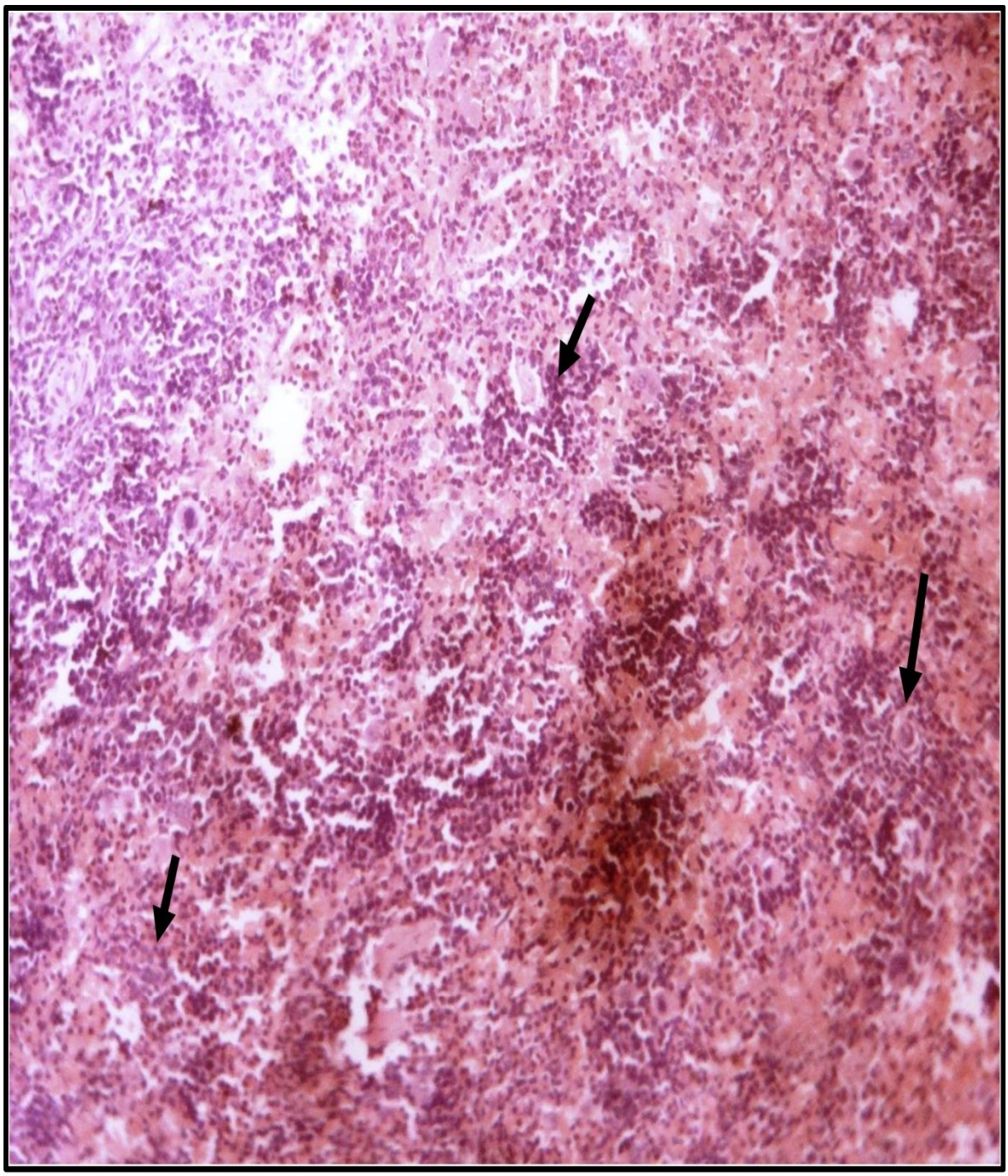

PLATE 10: Photomicrograph from Spleen section of rat in CYCLO control group showing marked immune depletion of white pulps [arrows] with most being hardly identifiable.
[STAIN: H& E] [Mag: x100]

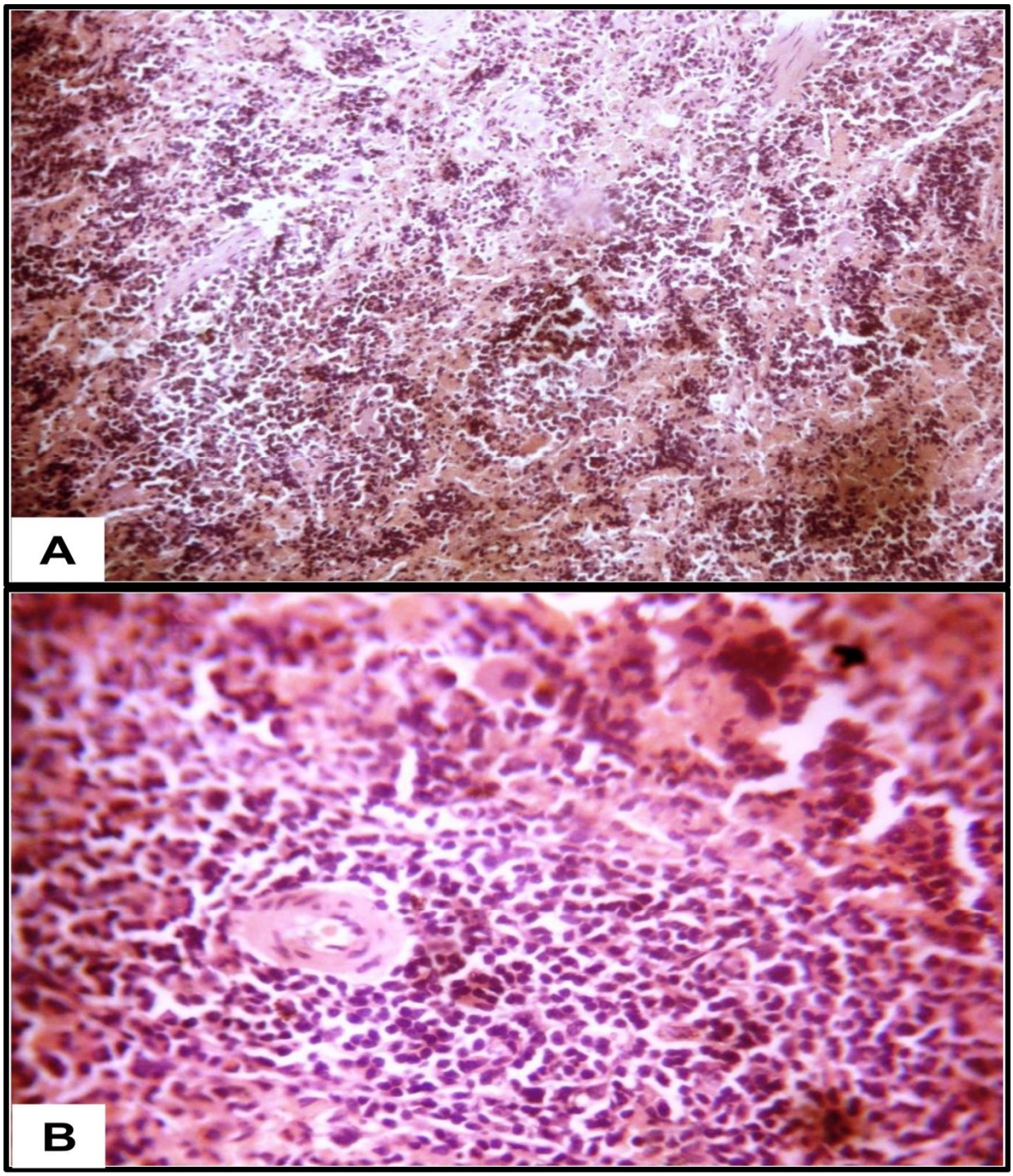

PLATE 11: Photomicrographs from Spleen section of rat in Group C treated with CYCLO and 500mg/kg b.wt. of AE. There is evidence of decreased spleen cellularity in both white and red pulps [A]. Slight immune depletion of the white pulp lymphoid follicle is shown [B].
[STAIN: H& E] [Mag: A – x100; B – x400]

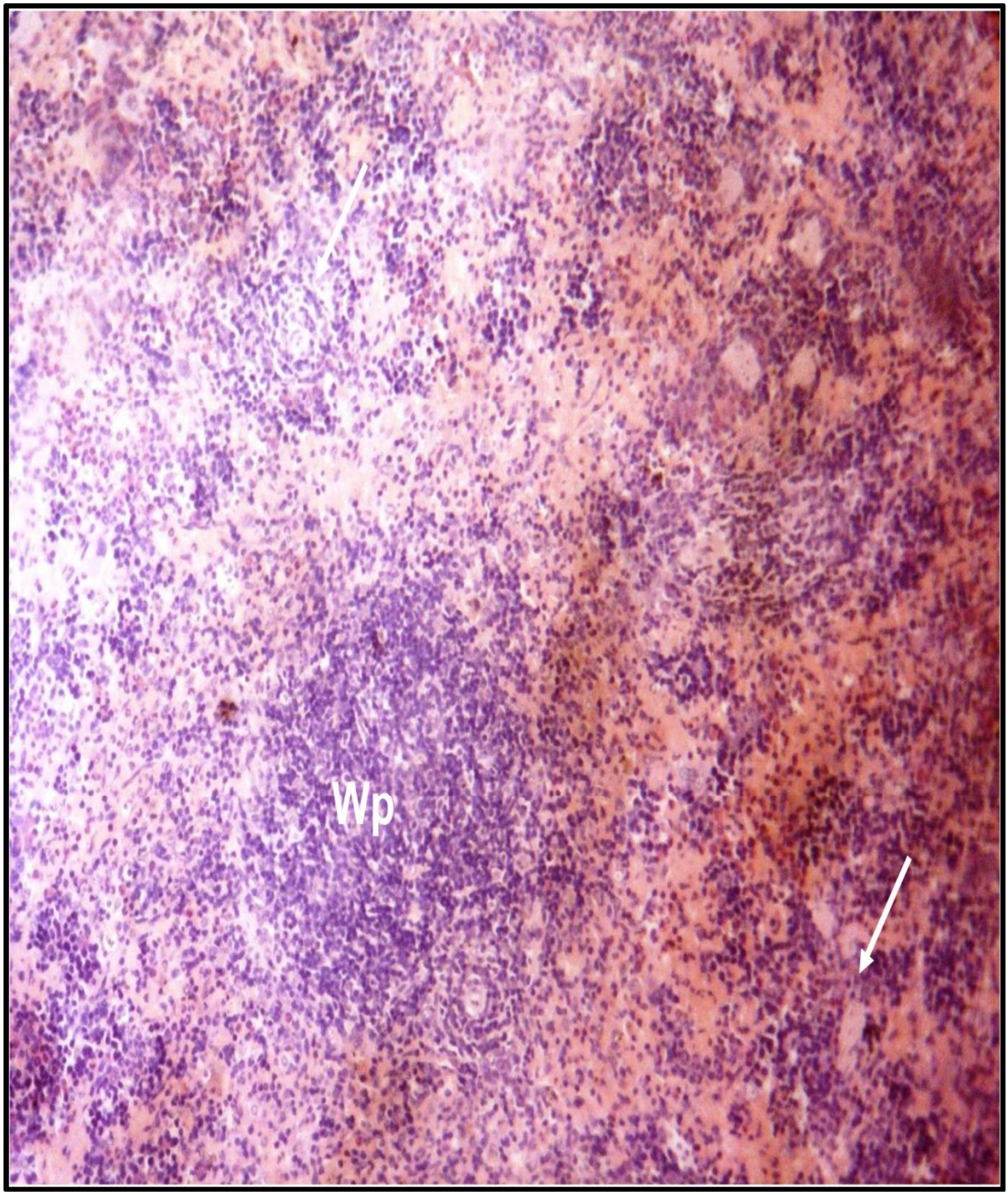

PLATE 12: Photomicrographs from Spleen section of rat in Group D treated with CYCLO and 1000mg/kg b.wt. of AE showing normal white pulp [Wp] and few appear mildly depleted [arrows].

[STAIN: H& E] [Mag: x100]

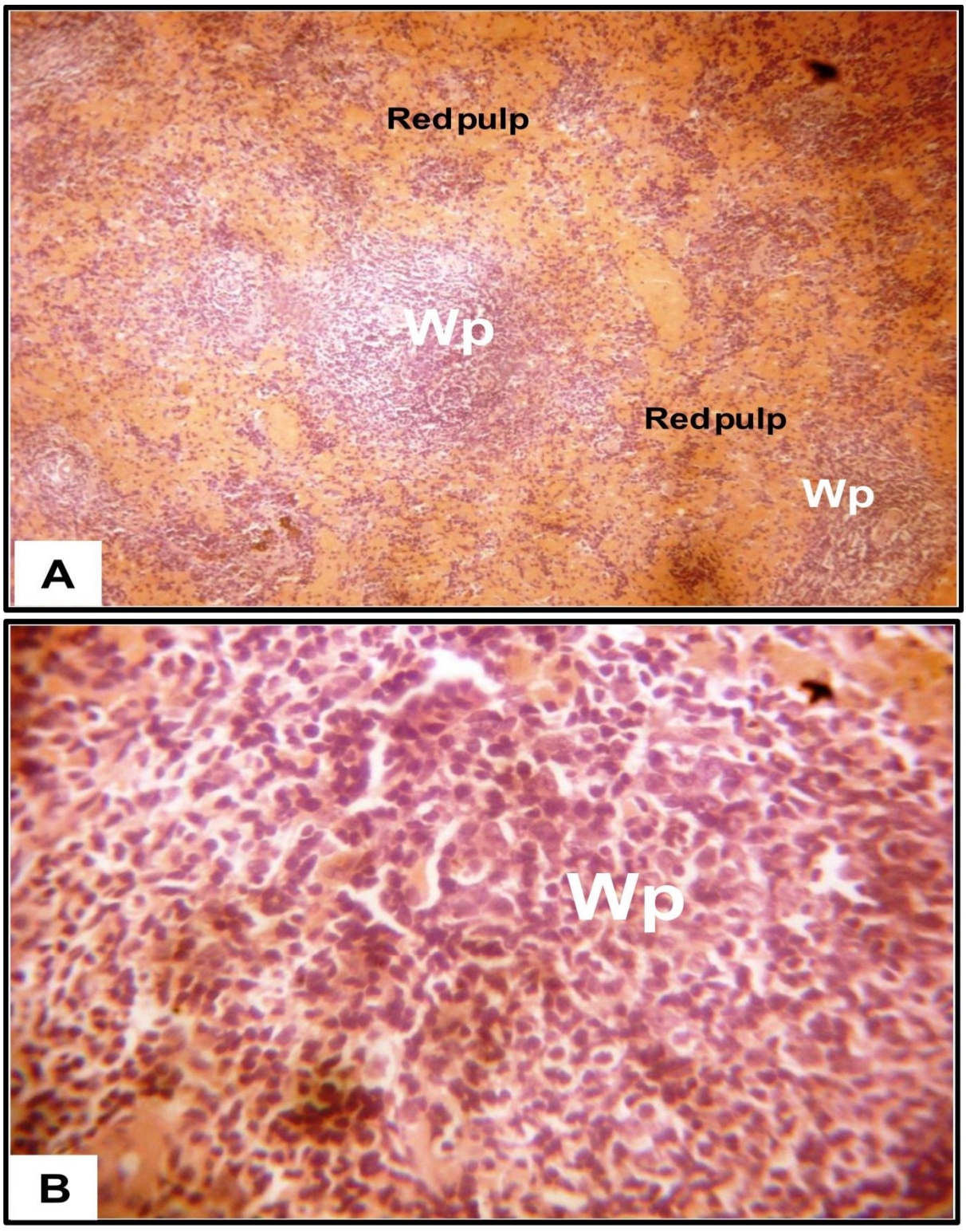

PLATE 13: Photomicrographs from Spleen section of rat in Group E treated with CYCLO and 500mg/kg b.wt. of ME showing intact cellular compartmentalization of the spleen. The cellularity of most white pulps [Wp] as shown in B appear normal.

[STAIN: H& E] [Mag: A – x100; B – x400]

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Ocimum tenuiflorum is a highly valuable herb, possessing many pharmacological and medicinal properties (Sai, *et al*, 2014). It was recognized thousands of years ago by ancient India as one of the greatest healing herbs (Ashwani, 2012). Medicinal values of *Ocimim tenuiflorum (tulsi)* include treatment of various ailments such as wound, common cold, headache, fever, cough, malaria, asthma, bronchitis, hepatic diseases, stress, genitourinary disorders, *Ocimum tenuiflorum* possess anti cancer, anti inflammatory, anti viral and antioxidant properties. It has immunomodulatory effect and lots of other medicinal values. The result of acute toxicity studies showed that *Ocimum tenuiflorum* had an oral LD_{50} > 5000mg/kg body weight in albino rats. The phytochemical analysis of the *Ocimum tenuiflorum* leaf extract reveal the presence of alkaloids, reducing sugars, terpenoids, flavonoids, carbohydrates, proteins, resins, tannins, saponins, steroids, glycosides, oils and acidic compounds. Alkaloids have pharmacological effects, they are used as local anesthetics, analgesic (Raymond *et al*, 2010), antibacterial, anticancer, antihypertensive, antimalaria, antitumor, antiprotozoal agent, antipyretic, anti asthma, vasodilator, nerve stimulant (Veselovskaya and Covalenko, 2000), muscle relaxant and cough medicine, (Hesse, 2002). Alkaloids are also used as insecticides and pesticides (Gyorgy *et al*, 2002). Flavonoids have many biological and pharmacological functions this include antioxidant (Cazarolli *et al*, 2008), anticancer, antibacterial (Cushnie Lamb, 2011), antifungal, anti viral (Friedman, 2007), anti allergic, anti-inflammatory (Yamamoto *et al*, 2001), anti diarrheal activities. Flavonoid inhibits coagulation, thrombus formation, and platelet

aggregation. It reduces the risk of atherosclerosis (Siasos, *et al*, 2013), arterial blood pressure and the risk of hypertension. Flavonoids also reduces oxidative stress, it modifies vascular inflammatory mechanism, blood lipid level modifier, improves endothelial and capillary function and regulate carbohydrates and glucose metabolism (Van Dam, *et al*, 2013). Glycosides play an important role in living organisms. Many plants store glycosides as inactive chemicals, such plants are later used as medications (Brito-Arias and Marco, 2007). Proteins are large biological molecules consisting chains of amino acid residue, they perform great functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. The chief characteristic of protein is its ability to bind to other molecules specifically and tightly. The best known role of proteins in the cell is as enzymes, which catalyze chemical reactions about 4000 reactions are catalyzed by enzymes (Bairoch, 2000). Tannin has antiviral (Lü, *et al*, 2004), antibacterial, (Akiyama, *et al*, 2001) antiparasitic effect (Kolodziej and Kiderlen, 2005). Souza et al discovered that tannin has anti-inflammatory, antiulcer antioxidant property, and also used to treat iron overload (Souza *et al*, 2006).

Effect of *Ocimum tenuifluorum* on immunossupressed albino Wistar rats was investigated in this research. The haematological analysis of the cyclophosphamide induced rats in Table 4.1 group B showed drastic decrease in neut, lym, mono, and eos count, indicating immunosuppression. The blood sample of the animal treated with methanol and aqueous extract of the *Ocimum tenuiflorum* showed great increase in neutrophil count, in groups C, D, E and F. We observed that high dose of methanol extract increased neutrophil count than aqueous extract this shows that methanol extracted more substance from the *Ocimum* herb than the aqueous. Aqueous extract also raised the neutrophil count in both low and high dose when

compared with the normal control and CP control. It is possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release it into the blood stream. Neutrophils are one of the arms of innate immune system (Alberts *et al*, 2002) Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms. The granules of the neutrophil contain many enzymes, which makes it a powerful and effective killer machine. This is because neutrophil is capable of ingesting and killing bacteria and provide defence against infections in the body, and hence, deficiency of neutrophils in the body leads to great defects, including conditions such as chronic granulomatous disease (Ofem *et al,* 2012). This effect on neutrophil count may be partly responsible for the claim that *Ocimum tenuiflorum* has antibacterial activities, since the two extracts increased neutrophil count and probably due to the presence of flavonoid, alkanoids, and tannin in the extract these bioactive agents have anti-bacterial effects.

We equally observed that the same methanol extract at lower dose Table 4.1 group E, caused high increase in lymphocyte count, increase was also recorded in the rats treated with aqueous extract in groups C and D when compared with the drug induced group, this could also be that *Ocimum* extract contain some compound capable of stimulating lymphopoiesis (Janeway *et al*, 2005) which could be as a result of bioactive compounds; alkanoids, flavoniods, tannin and saponin that have anti viral and anti cancer effects. Lymphocytes are the cells of the adaptive immune system. They produce circulating antibodies, B-lymphocytes and T-lymphocytes. B cells are involved in the humoral immune response, while T cells are involved in cell-mediated immune response. Both B cells and T cells carry receptor molecules that recognize specific targets. The killer T cells and the helper T cells recognize antigens coupled to class I and class II major histocompatibility

complex molecules (Holtmeier and Kabeitz, 2005).The cytotoxic T-cell attack and destroys cancerous infected cells or cells that have been infected by viruses (Middleton *et al*, 2002). With this reason *Ocimum* can be said to be an immume booster because it enhances the production of lymphocytes. High dose of methanol extract reduced lymphocytes count Table 4.1 group F. The reduction in lymphocyte count could probably be due to cell margination rather than low production (Dorland, 2007). Eosinophil count recorded increase in groups C and E, in Table 4.1 that received low dose of aqueous and methanol extract, groups C and D was also increased compared to the cyclophosphamide (CP) control. Whatever stimulated neutrophil production was also responsible for eosinophil production. Eosinophils are also one of the innate immune system. They secrete chemical mediators that are involved in defence against parasites and play a role in allergic reactions, such as asthma (Kariyawasam and Robinson, 2006). Monocytes increased in groups C and D, treated with aqueous extract, and there was slight increase in group F that received high dose of methanol extract. Monocytes are one of the innate immune cells, they are produced from the myeloid progenitor cells, after few hours in circulation they migrate to the tissues and transformed to macrophages where they perform specific functions in deferent tissues (Hoffbrand *et al*, 2006). Macrophages are important mediators in the activation of adaptive immune system they act as antigen presenting cells to T lymphocytes (Mayer Gene, 2006). There was an increase in eosinophil count in groups C and E that was treated with low dose of aqueous and methanol extracts. Eosinophils belong to innate immune cells, they attack foreign proteins and parasites, equipped with Fc and C3 receptors for antibody and complement proteins they bind and engulf opsonized antigens (Dailey, 2001). Total WBC (white cell count) was seen to be on the increase at low dose and high dose of aqueous and methanol extract administration Table 4.1 groups C, D, E and F when compared with the CP

induced group. On day 22 of this research Table 4.2 we observed an increase in all the leucocytes count in groups C, D, E, and F, treated with AE and ME, this indicate immune recovery. We also observed that the leucocytes count of the drug induced group B equally increased, this prove that the rats have recovered from the trauma inflicted to its immune system. Groups G and H was included in this study to check if there will be difference between the normal control and the healthy rats administered with the leaf extracts; we observed that there was obvious increase in neut count at low dose of AE but decreased count was observed at high dose of ME when compared with normal control, there was no changes in lymphocyte count at low dose of AE, GPG while increased count was recorded at high dose of ME, GP H when compared with the normal control Table 4.1. On day 22 Table 4.2 neut rather decreased at low dose of AE and increased at high dose of ME while lymphocytes decreased at low dose of AE and increased at high dose of ME. The extract at both high and low dose caused an increase in the erythrocyte count. This was confirmed by the increased hematocrit (PCV) and percentage Hb in the high-dose and low dose recipient group when compared with the CP control group. In normal circumstances, local tissue anoxia leads to the formation of a glycoprotein called erythropoietin, which stimulates increased production of erythrocytes. It is very likely that *Ocimmum tenuiflorum* leave extract contains erythropoietin-like agent(s) which are responsible for the increased production of erythrocytes (Ofem *et al*, 2012).

The histological profile of the liver and spleen of the normal control group showed normal features of hepatic tissues, normal central artery, red and white pulp (Plate 1 and plate 9). In the cyclophosphamide induced rats, the histological photomicrograph indicate serious injury to the hepatic tissues, showing marked ruptured central vein and inflammatory filtration at the periportal region, the white

pulp cannot even be identified in the spleen, indicating immune depletion, (Plate 2 and plate 10). In the treated groups D and E that received high dose of AE and low dose of ME, there was no obvious histopathological alterations on the liver tissues, the photomicrograph showing intact central vein and normal histoarchitecture of the hepatic tissues (plate 4 and plate 5) this indicate the healing effect of the leaf extract. The ruptured central vein, marked cellular infilteration and degenerative hepatocytes observed in groups C and F could be as a result of the injury caused by effect of the cyclophosphamide, which may have healed if the period of treatment was longer. Groups G and H that received extracts only (plate 7 and plate 8) shows no obvious hepatic changes, the portal tract, hepatocytes within the lobules and sinusoids are intact. The mild raptured central vein and mild cellular infiltration in group H may have occurred during metabolism of the leaf material and detoxification process by the liver. High dose of AE and low dose of ME showed normal white pulp, intact cellular compartment of the spleen (Plate 12 and 13) suggesting recovery from immune depletion. The activity of this *Ocimum* was believed to be due to its active phytochemicals which do not work in isolation.

5.2 CONCLUSION

In conclusion, this research had revealed that the *Ocimum tenuiflorum* leaf extract may have the ability to boost suppressed immunity since it increased the values of the two main immune cells, lymphocytes and neutrophils in immunosuppressed rats. *Ocimum* equally confirmed its hepatoprotective and splenoprotective effect as shown by the histology analysis of liver and spleen, therefore it is safe for medicinal use.

RECOMENDATION

I recommend that people with suppressed or low immunity should use *Ocimum tenuiflorum* leaf in their daily meal preparation so as to boost their immunity.

Ocimum tenuiflorum extract can be included in the preparation of immune boosting drugs. Further studies should however extend the duration of the extract administration to check whether there will be adverse effects in liver, spleen and the other organs of the body.

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Akiyama H., Fujii K., Yamasaki O., Oono T., Iwatsuki K. (2001). ñantibacterial action of several tannin against staphylococcus aureusò Journal Antimicrob. Chemother. **48** (4): 487ï 91.

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APPENDIX I

MATERIALS AND EQUIPMENT

Oral cannula for oral intubation of the leaf extract.

5 ml EDTA (Ethylenediamine tetracetic acid) anticoagulant bottles

Capillary tubes

Weighing balance

Cotton wool

Refrigerator

Ocimum tenuiflorum leaves

EQUIPMENT: AUTOMATED MACHINE (MYTHIC 22)