TITLE PAGE

MORPHOLOGICAL EFFECT OF CRUDE EXTRACT OF VISCUM ALBUM LINNAEUS ON SOME SELECTED VISCERAL ORGANS OF ALBINO RAT

AN M.Sc. DISSERTATION

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CERTIFICATION

DEDICATION

I dedicate my project work to Almighty God, my way maker and giver of life. A special feeling of gratitude goes to my husband, Mr. Sylvester Azubuike who is a standard for hard work and diligence.

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ABSTRACT

The morphological effects of crude extract of *Viscum album Linnaeus* called ñAnwusiò in Ika dialect on some selected visceral organs of albino rat was investigated for this study. Seventy (70) male and female adult albino rats of about 2 - 4 months old and 180 - 200g body weight were used for the study. Forty (40) rats were used for acute toxicity testing and thirty (30) for this study. The albino rat where randomly selected into Six (6) groups of 5 rats per cage and labeled as cages 1, 2, 3 and 4 and cages 5 and 6 severed as control groups. Tap water and standard pellet (Guinea Feed) were given to animals throughout the duration of the study. Cage 1, 2, 3, and 4 were treated with the leaf extract in order 100, 150, 200 and 300mg/kg body weight with regards to the LD_{50}. Cages 5 and 6 were without experiment dose. The doses were orally administered for 28 days. At the end of 28 days, the selected visceral organs such as; oesophagus, duodenum, pancreas, gall bladder, liver and kidney were taken, processed and stained histologically. The tissue were stained with haematoxylin and eosin, photomicrograph was done. Histology of the liver and kidney, the extract induced mild periportal inflammation with haemorrhagic necrosis in the liver and tubular necrosis in the kidney. In the G.I.T, it induced mild mucosal inflammation, with increase dose it induced mild glandular hyperplasia. In conclusion, *Viscum album* leave extract was not well tolerated. Prolonged administration of leave extract will cause serious life threatening undersirable effect. Particularly, in the liver and kidney, it may lead to destruction of the tissue as well as loss of renal mass.

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ABBREVIATIONS

VT - Vicotoxin

V - *Viscum*

QoL - Quality of Life

AT - Advanced Treatment

ADRs - Adverse Drug Reactions

ISC - Iscador

CRC - Cancer Research Campaign

DFS - Disease Free Survival

CAM - Complementary and Alternative Medicine

CRF - Cancer Related Fatigue

STZ - Streptozoticin

L - Loranthaceae

LDL - Low Density Liproprotein

HDL - High Density Liproprotein

AM - Africa Mistletoe

ML - Mistletoe Lectins

RBC - Red Blood Cell

LP - Lamina Propria

CT - Connective Tissue

PHC - Primary Health Care

ORAC - Oxygen Radical Absorbance

TEAC - Trolox Equivalent Antioxidant Capacity

FRAP - Ferric Reducing Ability of Plasma

GIT - Gastrointestinal Tract

WHO - World Health Organization

LD - Lethal Dose

GB - Gilibenilamide

DNA - Deoxyribonucleic acid

CHAPTER ONE

1.0 INTRODUCTION

People on all continents have used thousands of indigenous plants for treatment of ailment since prehistoric times. By watching animals, these ancient people learned what herbs and plants would work for various condition The use of plants as medicine predates written human history; but the earliest archaeological evidence indicates that a 60,000 year ï old Neanderthal burial site yielded large amount of pollen from plants that were later known to have been used in herbal remedies. The written record of herb dates back over 5,000 years to the Sumerians, who described well ï established medicinal uses for such plants as laurel, caraway, and thyme. Ancient Egyptain medicine of 1000 B.C are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicine. The Old Testament of the Bible also mentions herbs use and cultivation of such plant as mandrake, vetch, caraway, wheat, barley, and rye. Herbalism, sometimes referred to as folk medicine, herbal medicine, and herbology, not only utilizes plant, plant extract, but also fungal and animal products, mineral, and shells. Today, herbs are available in tablets, capsules, powders, teas, extracts and fresh or dried plants. Many of these can be very beneficial, however, cautions should be taken when utilizing these powerful supplements. Some can actually cause health problems, especially as they interact with other drugs. It is recommended to consult your doctor before using herbal supplements, always follow lab directives, and be especially

cautious if you are pregnant or nursing. The 21st century is witnessing serious scientific effort to discover major active ingredients in medicinal plants through research and development. This perhaps may be sequel to the inability of orthodox medicine to comprehensively address most disease conditions plaguing mankind or it may be a response to the clarion call by the World Health Organization that developing countries should endeavor to develop and utilize local medications that are most appropriate to their local circumstances especially for Primary Health Care (PHC) in order to cut down on huge cost associated with incessant drug importation (WHO, 1996). However, in the academia, the widely held view for the sudden rise in research work in herbal medicine has been linked to the quest to meet research mandates and perhaps some routine promotion. Whatever reason that may be ascribed to increase in research and development activities in medicinal plants, one thing is instructive which is that herbal medicine is beginning to take the center stage in the management of health ï related problems in the Nigerian health care system. Continuous research and development may be on the increase due to incessant changes in and the quest for new technology especially in this era of globalization.

Through technological research and development, countries of the Far East Asia as China, South Korea and India have been able to develop and upgrade the quality of their herbal medicine. China for instance is the World leading producer and exporter of herbal medicine. In 1993 the total sales of herbal

medicine in China amounted to $2.5 billion. In Japan there has a 15 fold increase in herbal medicine sales when compared to 2.5 fold increase of pharmaceutical product between 1974 and 1989 (WHO, 1996). The countries of the Far East Asia have been able to meet 75% of their health care needs through the development and utilization of herbal medicine and traditional medicine practice.

Meanwhile, only 40% of herbal medicines consumed in Nigeria are produced locally and remaining 60% are imported from foreign countries. Again, the commercial interest to mass produce herbal medicine through research and development and make them available for local and international use is lacking (Ohuabunwa, 1998) and would probably take a longer time for this to improve and grow significantly. In Nigeria, effort at local herbal medicine research and development is yet to yield the desired benefits due to low funding of research and development activities, lack of adequate infrastructural facilities, non - commercialization of most research result, low demand that constraint research and development investment and problems faced by herbal medicine researchers (Oyeleran ï Oyeyinka, 1996; Ilori, *et al,* 2005).

Plant 1.0: Photograph of *Viscum album* plant called ñanwusiò in Ika dialect.

Source: Mistletoe in Cedar Elms Turpin Park Highland Village.

Plant 2.0: Photograph of *Viscum album* leaf called ñanwusiò in Ika dialect.

Source: Mistletoe in Cedar Elms Turpin Park Highland Village.

1.1 HISTORY OF MISTLETOE

Mistletoes has been used in a number of indications during the known history. Hippocrates of Cos (460-370 BC) used mistletoe to treat diseases of the spleen and complains associated with menstruation. Celsus (25 BC ï 50 AD) also describes using mistletoe in the fifth book of De Medicina. He mixed it with various organic or inorganic substances to create plasters and emollient, which he then used to treat abscesses, carcinoids, and scrofula. There is also evidence that Pliny the Elder (23-79 AD) used mistletoe to infertility and ulcers. Alexandrian a physician and surgeon Paulus Aegineta (625-690 AC), and the Persian philosopher and physician Avicenna (Ibu-Sina, 980-1037 AC) were also familiar with mistletoe although it is not clear how they employed it in treatment (Foy, 1887). Apparently, 18th century onwards mistletoe found application in management of ñweakness of the heartò and oedema. The homeopathic material medicine still indicates uses of tincture of *Viscum album* today (Boericke, 1992). Pliny the Elder (23-79 AC) the legendary roman naturalis and author of Historica Naturalis reported that the Druids followed ceremonious removal of mistletoe growing on oak trees using a golden sickle on the sixth day after new moon. They considered the plant to argument fertility and an antidote for poisons. They believed it to possess miraculous properties to cure every illness due to its all healing abilities. During the middle ages Mistletoe alone or in combination with other aromatic substances was used as incenses allowed one to get in control with ñelementary power of natureò and to find the ñinner stabilityò (Ratsh, 1997). Theophrastus (371-189 BC) described

mistletoe in his botanical treatise Historia Plantarum as an evergreen plant growing in pine and fir trees, used for feeding animals during harsh winters. He acknowledged that mistletoe does not grow on earth, and it seeds are spread through birds excreta that feed on mistletoe berries. Celtic Druids considered mistletoe sacred primarily because of the ability of this plant to remain flourishing green even in dead winter without having roofs on the earth. They considered this plant a symbol of ever-lasting life.

1.2 AIM AND OBJECTIVE

The general aim of this study is to assess the effect of consumption of crude extract *Viscum album* on some selected visceral organs of albino rat. The specific objectives is to:

1. Determine the oral median lethal dose (LD_{50}) of the leaf extract of *Viscum album.*

2. Investigate the phytochemical composition of the leaf extract.
3. Investigate the impact of consumption of *Viscum album* leaf on oesophagus, duodenum, pancreas, gall bladder, liver and kidney of albino rat.

CHAPTER TWO

2. 0 LITERATURE REVIEW

The stem is yellowish and smooth, freely forked, separating when dead into bone like joints. The leaves are tongue - shaped, broader towards the end, 1 to 3 inches long, very thick and lengthy of a dull yellow - green colour, arranged in pairs, with very short foot stalks. The flowers, small and inconspicuous, are arranged in threes, in close short spikes or clusters in the forks of the branches,

and are of two varieties, the male and female occurring on different plants. Neither male nor female flowers have a corolla, the parts of the fructification springing from the yellowish calyx. They open in May. The fruit is globular, smooth, while berry, ripening in December.

Birds like Mistle thrush and blackcap that feeds on mistletoe berries normally propagate mistletoe. These birds remove the seeds along with mesocarp from the fruit and bring it in contact with sites on the host trees. These seeds firmly attach to the branches when gum like mesocarp dries out along with the seed. If the climate is not suitable (too cold) the embryos may not germinate immediately, after March embryo grow and elongated hypocotyl may be seen. The hypocotyl epidermal cells secrete a viscous liquid that helps the new mistletoe attach itself directly to the host tree. In addition to mechanical forces, enzymes are released by mistletoe that probably aid opening up of the host tissue. The tip of the hypocotyl broadens to a flat disk, the so called holdfast, and papillae connected to the host are draw to the periphery, opening up the host periderm layer by layer. The slightly oval holdfast shows bilateral symmetry, including a meristematic zone along the major axis and adjacent to the host bark. With cell division starting from here, the meristematic tissue is penetrating into the opened up host periderm. Thus mistletoe attaches itself to the host to derive necessary nutrients for its survival. Mistletoe is always produced by seed and cannot be cultivated in the earth like other plants, hence the ancients considered it to be an excrescence of the tree by rubbing the berries

on the smooth bark of the underside of the branches of trees till they adhere, or inserting them in clefts made for the purpose, it is possible to grow mistletoe quite successfully, if desired. Mistletoe is found in Australia, Africa, America, Canada and Europe. Although a number of mistletoes are found in Europe the two most widely recognized are *Viscum crucitatum ex Boiss* and *Viscum album Linnaeus*, the later has been studied extensively and has become almost synonymous to European mistletoe. *Viscum crucitatum* is a poisonous species of mistletoes, *Viscum crucitatum* is native to South Spain and East Portugal. In addition to Spain and Portugal, it is also found in Australia, North Africa and Asia. The most striking features of this mistletoe are its red berries that are 6 to 10 mm in size, hence the plant is also called red berried mistletoe. The stem of *Viscum cruciatum* is up to 60cm long, yellowish green. Leaves are 2 ï 4 cm long and 1 to 2 cm broad, often whorled, obovate ï oblong, obtuse, yellowish green, cymes shortly pedunculate.

Mistletoes are a group of obligate semi-parasitic plants that belong to the families Viscaceae and Lorathaceae, both these plant families are taxonomically related and are part of the order Santalales. The Viscaceae family is composed of seven general namely, *Arcenthobium, Ginalloa, Dendrophthora, Phoradendron, Korthalsella, Notothixox, Viscum*. There are over hundred know species across the globe. The most notable and studied species of Mistletoe is *Viscum album Linnaeus*, the famous European White-berry mistletoe. *Viscum album Linnaeus*, was one of the many species of Mistletoes originally described

by Linnaeus. Itôs an evergreen plant that grows semi-parasitically on the stem of its host by deriving water and minerals from it. A number of mistletoes are found on African continent, however, most prevalent are the mistletoes belonging to Genus *Viscum*. Around 45 species in Africa and another 30 in Madagascar have been reported. Pohil and Weins in 1998 provisionally divided the African mistletoes in eleven groups based on features exhibited by the species.

2.1 MEDICINAL USES OF AFRICAN AND EUROPEAN MISTLETOE

Viscum fisheri and *Viscum tuberculatum* have been used in Kenya as poultice on chest for pneumonia. *Viscum tuberculatum* have been employed for liver troubles. In southern Africa both African and European cultures employ *Viscum capense* and *Viscum rotundifolium* for medicinal purposes. European uses these plants to remove warts. *Viscum capense* has also been used for bronchial problems, as astringent and as blood coagulant. Teemohlware a herbal tea made using *Viscum rotundifolium* is believed to cure heart ailment and purify blood (Oliver, *et al,* 1987; Polhill, *et al,* 1998).

Mistletoe preparations used therapeutically are complex plant extracts containing a wide variety of substances ranging from high molecular height compounds such as flavonoids and others. Few noteworthy chemical entities identified in mistletoe extracts that are worth mentioning are thionins (viscotoxic), carbohydrateï binding proteins (lectins), flavonoids, alkaloids,

phenylpropanoids, phytosterols, triterpenes, polyalcohols, monosaccharides, oligo-and polyaccharides.

Certain lectins found specifically in mistletoe extract collectively called as Mistletoe Lectins (ML) are subject of evaluation for immunomodulation and cytotoxic effect of mistletoe extract obtained from *Viscum album*. The content of proteins, polypeptides and carbohydrates in mistletoe extract is effected by the host tree and other natural factors like sex, local climate, harvesting time, parts and age of plant used etc (Hincha, *et al,* 1997). In traditional medicine mistletoes, in East Asia, are employed as analgesic sedative, spasmolytic, cardiotonic and anticancer agent; the herbs are also used to strengthen tendons and bones, tone the liver and kidneys, expel pathogens associated with rheumatism, stabilize the fetus and cause lactogenesis (Zee Cheny, 1997). Zulus used *Loranthus* and *Viscum* subspecies as enema for stomach troubles in children. *Viscum aethiopicum* was a remedy to treat diarrhoea. Nigeria folk medicine practitioners employed Loranthus Bengwensis Linnaeus, to treat diabetes mellitus (Bikomo, *et al,* 1994). While the European mistletoe has been studied intensively, we know less about East-Asian populations of mistletoes as therapeutic herbs, especially in the light of modern medicinal approach. However, as percent studies, *Viscum album var coloratum* growing in Korea shows similar cytotoxic and immunological activities as compared those of European mistletoe. Extracts from *Viscum album Var. coloratum* have inhibitory effects on tumor angiogenesis and metastasis (Won ï Bong Park,

2000).The effects of Nigerian specie of *Viscum album Linnaeus* leaf extract on the blood pressure of normotensive and doca-induced hypertensive rats was investigated (Eno, *et al*, 2004). Infact, herbal medicine still remains the first line of medication amongst a vast majority of Africans (Barbara and Peter, 1992). The mistletoe leaf extract is said to possess antidiabetic (Obatomi, *et al*, 1994) immunomodulatory (Solar, *et al,* 1998), bacteriostatic (Fulder, 1998) and therapeutic values for many other ailments. Investigations aimed at providing the scientific basis to the hypotensive properly of mistletoe leaf extract have already been provided (Obatomi, *et al*, 1994; Hajto, *et al*, 1999; Lavastre, *et al*, 2002). The resultant effect of the crude mistletoe extract reduces the mean arterial blood pressure of both the normotensive and hypertensive rats. It is very likely that this action is achieved by a reduction in heart rate and probably other mechanisms as well. This is consistent with other reports about the activity of the mistletoe on blood pressure. However, that the mistletoe employs the autonomic pathways (adrenergic and cholinergic) in depressing the heart rate is very unlikely (Eno, *et al*, 2004).

Different species of *Viscum* are capable of parasitizing a large number of host species (Barney, *et al*, 1998). Meanwhile the antioxidant activity of European mistletoe (*Viscum album*) has been investigated (Visas, *et al*, 2009a). In recent years, antioxidants derived from natural; recourses, mainly from plants have been intensively used to prevent oxidative damages. Natural antioxidants have also some advantages over synthetic ones, being obtained easily and

economically and have slight or negligible side effects. Aqueous extracts of the European mistletoe have been widely used for decades as alternative treatment and adjuvant cancer therapy, particularly in Germany, Austria and Switzerland. The European mistletoe extracts are used in an adjuvant cancer therapy because of their immunostimulatory and simultaneously cytotoxic properties. These effects are usually more evidence for the whole extracts than for purified mistletoe lectins and viscotoxins alone (Eggenschwiler, *et al*, 2007). Mistletoe can biosynthesize their own compounds, but it can take some nutrients from the host trees. It has been suggested that pharmacologically achieve compounds may pass from the host trees to the parasitic plants (Bussing and Schnitzel, 1999). The main ingredients of the *Viscum album* extract are its three ribosome inactivating protein or lectins (mistletoe lectins, ML) ML-1, ML-2, ML-3 (Hajto, *et al*, 2005), the glycoprotein binding with D-galactose and N-acetyl-D-galatosamine, vicotoxin (VT) (Urech, *et al*, 2006) as well as, Oligosaccharides and polysaccharides, alkaloids (Khwaja, *et al*, 1980). The flavonoid patterns of *V. album* form various hosts were investigate by (Becker and Exner, 1980). They identified quercetin and a series of quercetin methyl ethers, which may be assumed to be accumulated on the plant surface. The epicuticular material of the *V. album* contains preferably the flavonol quercetin and its methyl derivatives, occasionally also the flavonol kaempferol and some of its methyl derivatives, and rarely the flavanone naringenin (Haas, *et al*, 2003). In recent year, the research studies were focused on the antioxidant activity of mistletoe (Onay-

Ucar, *et al*, 2006; Leu, *et al*, 2006; Yao, *et al*, 2006; Shi, *et al*, 2006; Viscas, *et al*, 2009b; Choudhary, *et al*, 2010).To evaluate the antioxidant capacities of plant extracts, numerous in vitro methods have been developed. DPPHC 2,2 diphenyl-1-picrylhydrazyl), ORAC (oxygen radical absorbance capacity, Trolox equivalent antioxidant capacity (TEAC), and Ferric-reducing ability of plasma (FRAP) are among the more popular methods that have been used (Wu, *et al*, 2004). The advantages and disadvantages of the method have fully been discussed in (Cao and Prior, 1998, Frankel and Meyer, 2000; Prior and Cao, 1999; Sanchez-Moreno, 2002). From the result, bioactive compound and the antioxidant activity are present in leaves and also in stem in all the mistletoes samples examined (aqueous and ethanol) (Vicas, *et al,* 2009b).

Meanwhile a systematic evaluation of the clinical effects of supportive mistletoe treatment within chemo-and/or radiotherapy protocols and long term mistletoe application in non-metastatic colorectal carcinoma (Walter, *et al*, 2009). In Germany a Switzerland supportive therapy in patients with CRS and many other solid tumors is frequently accompanied by treatment with European mistletoe (*Viscum album*) extracts, with the intention to reduce the AT-ADRs, to improve the QoL, and to prolong survival. Unfortunately, published relevant clinical data are rather sparse, consisting mainly of case reports, small clinical studies, and only a few controlled trials which often show contradictory or inconclusive results and frequently suffer from methodological weakness (Horneber, *et al*, 2008). The result from the investigation suggest a beneficial

effect of supporter care of mistletoe extract Iscador (ISC) therapy within AT protocols and long term ISC treatment in stage I-III CRC patient, particularly improvement in AT-ADRs and symptoms and possible extensions of DFS (Walter, *et al*, 2009).

Another investigation was done on mistletoes treatment in cancer-related fatigue (Kathrin Wode, *et al*, 2009). Cancer patients often we complementary and alternative medicine (CAM). Among the most frequently applied CAM therapies for cancer are aqueous extracts from European mistletoe (*Viscum album*), originally developed as a cancer remedy in the context of anthropsophic medicine (AM) (Kienle, *et al,* 2003; Kienle, *et al*, 2007). Mistletoe extracts show highly effects especially through induction of apoptosis, but they also possess DNA-stabilizing properties in mononuclear cells, they stimulate the immune system (in vivo and in vitro activation of monocytes/macrophages, granulocytes, natural killer cells, T-cells, induction of variety of cytokines) and can enhance endorphins (Kienle, *et al,* 2003; Bussing, 2000).

Injected in tumor-bearing animals, they display growth-inhibiting and tumor-reducing effects (Kienle, *et al*, 2003; Bussing, *et al,* 2000) Mistletoe remedies, either alone or in combination with surgery, chemotherapy, radiation or hormone therapy are applied in all cancer types and all stages of disease, in order to improve QoL and general condition, to reduce side effects of oncological treatment and to improve immunosuppression, and prolong time of progression and survival (Kienle, *et al*, 2003) controlled clinical trials found

best evidence of efficacy in relation to improvement of QoL and reduction of side effects of chemotherapy and radiation. Most trials also observed survival benefit, but not beyond critique. Mistletoe generally well tolerated, with no or only minor side effects (Kienle, *et al*, 2007). Mistletoe-prescribing physicians often observe a market improvement in fatigue after some months of mistletoe application, and even use fatigue as an indicator for individual adjustment of dosages. Nevertheless, CRF has not so far been addressed as a primary objective in mistletoe studies (Kienle, *et al*, 2003; Kienle, *et al*, 2007). For instance, two studies evaluating in-patient cancer treatment in AM hospitals (including mistletoe therapy) observed a significant improvement in fatigue levels (Carisson, *et al*, 2004; Heusser, *et al*, 2006).

Mistletoe treatment showed as effect on the severity of (Cancer-related fatigue). Symptoms as CRF is a major complaints in cancer patients for which few therapeutic options are available, these observations should be investigate further. Possible effect of multimodal treatment approaches should be clarified (Kanthrin Wode, *et al*, 2009).

An investigation was done on the metabolic extract of African mistletoes (*Viscum album*) on carbohydrate metabolism and hyperlipidemia in streptozotocin - induced diabetic rats (Oluwatosin, *et al*, 2012). Mistletoes have been used in the treatment of many diseases in traditional medicine in Africa. It has been reported to be effective in the management of chronic metabolic disorder (Ojewole, *et al*, 2007). A number of biological effects, such as

anticancer, antimicrobial, apoptosis-inducing and immune-modulatory activities have been reported for mistletoe (Onay, *et al*, 2006). Mistletoe teas and infusions are used for prevention and management of stroke in Nigeria, and it is used to improve the circulatory system and heart functions (Chaulya, *et al*, 2011). However, there is death of scientific data to justify and support the use of mistletoe in folkloric medicine. Hence the study to investigate the effect of African mistletoe on carbohydrate metabolism and lipid profile of streptozotocin - induced diabetic rats. Male Wistar rats, 220 ï 230 g were used for the study. Streptozoticin (STZ) intoxication caused a significant increase (P > 0.05) in the serum total cholesterol and triglycerides of untreated diabetic animals when compared with normal. Precisely total cholesterol and triglycerides were increased by 56% and 65%, respectively in untreated diabetic rats. Furthermore, serum LDL - cholesterol level of untreated STZ - diabetic rats increased by 75% relative to normal. In constrast, untreated STZ - diabetic rats had significantly (P > 0.05) lowered serum HDL-cholesterol level when compared with normal. Administration of African Mistletoe (AM2) significantly reduced (P > 0.05) the hypercholesterolemia and hyperglyceridemia in the diabetic rats. Also, AM2 significantly (P > 0.05) reversed the adverse effect of STZ on HDL-cholesterol levels in the animals. The reversal effect of AM2 on the lipid profile of STZ - diabetic rats was much stronger than Glibenilamide (GB) treated diabetic rat. It can therefore be suggested that African mistletoe may improve lipid profiles directly or

indirectly through reducing blood glucose in diabetic animals is observed in the study (Oluwatosin, *et al*, 2012). Apparently, the investigation of insulin - secreting activity of the traditional anti-diabetic plant *Viscum album* (Gray, *et al*, 1999). *Viscum album* (mistletoe) is a member of the Loranthacaea family. It has been reported to have a number of medicinal properties including the ability to lower blood pressure, slow the heart beat, stimulate the immune system, relax spasms and exert sedative, diuretic and anti-cancer (Bown, 1995). A tea prepared from leaves of mistletoe is used traditionally to treat diabetes in the West Indies (Peter, 1957). This treatment has been shown also to relieve the diabetic symptoms of severely hyperglycemic streptozoticin-diabetic mice, including polydipsa, hyperphagia and body weight loss (Swanston ï Flatt, *et al*, 1989). An antidiabetic could exert a beneficial effect in the diabetic situation be enhancing insulin secretion and or by improving/mimicking insulin action (Gray and Flatt, 1997a). This study was undertaken to investigate the possible presence natural product(s) in an aqueous extract of mistletoe which stimulates insulin secretion. A study provides evidence for the first time for water soluble, heat-resistant insulin reversing components in mistletoe. This in addition to representing an advocated plant treatment for diabetes, mistletoe represents a source of potential new oral hypoglycemia agent(s) as yet to be isolated and identified (Gray, *et al*, 1999).

However, the immune-system stimulating and cytotoxic properties of mistletoe have been investigates in labouratory and animal studies. Viscotoxins and

lectins have been investigated as active compound in mistletoe however, most research has focused on the lectins (Gabius, *et al,* 2001; Lenartz, *et al*, 2000; Stever-Vogt*, et al*, 2001; Goebell, *et al*, 2002; Kunze, *et al*, 2000; Mengs, *et al*, 2000; Burger, *et al*, 2001). Purified mistletoe lectins have demonstrated cytotoxic and immune system stimulation activities. To date, four different lectins: ML-I, ML-2, ML-3 and *Viscum album* chiti-binding agglutinin have been identified a mistletoe extract. ML-1 or Viscumin may be responsible for many of mistletoeôs biological effects. When a labouratory method was used to selectively deplete ML-1 from *Viscum album* extracts their cytotoxic and immune ï system stimulating properties were markedly reduced (Janssen, *et al*, 1993; Beuth, *et al*, 1995). It should be noted that fermentation eliminates most of the ML-I in mistletoe extract (Wagner, *et al*, 1986). Polysaccharide and oligosaccharide component of mistletoe extracts with substantial immune-stimulating properties (Stein, *et al*, 2002; Lyu, *et al*, 2004).The molecular structure of ML-1 consist of an alpha chain which can be seperated from one another (Timosehenko, *et al*, 1993; Dietrich, *et al*, 1992). Each chain type appears to mediate a subset of the activities described for the intact lectin. Cytoxicity is associated mainly with the alpha chain. In labouratory studies, the ML-1 alpha chain has coupled to monoclonal antibodies to produce immunotoxins that target and kill specific cell types (Wiedloch, *et al*, 1991; Tonevitsky, *et al*, 1991). However the phytochemical screening of Tapinanthus dodoneifolius (DC) Danser called ñKauchiò in Hausa a species of African

mistletoe, showed the presence of anthraquinones, saponins, tannins (Deani and Sadiq, 2002). European mistletoes has gained widespread research. The primary chemical constituents have been found to vary according to the host plant but typically include glycoprotein, polypeptides (viscotoxin), flavoniods, flavonol agylcones (methyl ethers of quercentin kaemforal), lectins such as *V.album* agglutinin I, II and III, triterpenes, saponins, caffeic acid, lignans, choline derivatives related to acetylycholine, vitamin C, histamine, resins thionins, cardionolids, and phenolic compounds (Edlund, *et al*, 2000; Wollenweber, *et al*, 2000; Lyu, *et al,* 2000). A decoction of the leaves of mistletoe is traditionally used in the treatment of hypertension and to alleviate symptoms of hypertension such as headache, dizziness, palpitation, etc (Fernanden, *et al*, 2002; Deliorman, *et al*, 2002). It has been observed that methods used by traditionalist in early diagnosis lack scientific basis (Oyebola, 1980).

2.2 HISTOLOGY OF THE ORGANS UNDER INVESTIGATION

2.3 DUODENUM

The small intestine is 4-6 metres long in humans. To aid in digestion and absorption the small intestine secretes enzymes and has mucous producing glands. The pancreas and liver also deliver their exocrine secretions into the duodenum. The mucosa is highly folded, large circular folds called plicae circulares are most numerous in the upper part of the small intestine. The lining columnar epithelial cells have fine projections on their apical surface called

microvilli. Together, these folds provide a huge surface area for absorption. Between the villi there are crypts, called crypts of Lieberkuhu, which extend down to muscularis mucosae. These crypts are short glands. The lamina propria which underlies the epithelium has rich vascular and lymphatic network, which absorbs the digestive products, and there is a muscularis mucosae layer immediately at the base of the crypts. The lymphatic capillaries are called lacteals and absorb lipids. The vascular capillaries are fenestrated to aid absorption. The muscularis external layer contains two layers of smooth muscle, an inner circular and outer longitudinal, for continuous peristaltic activity of the small intestine. There are around 200 or so lymphoid aggregations called Peyerôs patches in the mucosa. Lymphoid aggregations are commonly found in the sub-mucosa of the small intestine as you can see one here. The epithelium of the villi is made up of tall columnar absorptive cells called enterocytes and goblet cells, which secrete mucin, for lubrication of the intestinal contents, and protection of the epithelium.

The first part of the small intestine is the duodenum, and its structure is similar to that seen elsewhere in the small intestine, with some differences. The villi are broader. Peyers patches are less common, and it has are unique feature: Brunnerôs glands, which is found in the submucosa. Both Brunnerôs glands, and the goblet cells in the duodenum secrete mucus. The mucus secreted by Brunnerôs glands is alkaline, and helps to neutralize the acid chime produced by

the stomach, to produce chime with a pH suitable for the digestive enzymes of the small intestine.

2.4 GALL BLADDER

The gall bladder is a specialized portion of the bile duct. The gall bladder is shaped like a small sack to store bile, it has an absorptive epithelial lining to concentrate bile, and it has a muscular wall to expel bile. In its absorptive function, the gall bladder epithelium is a highly exaggerated striated duct, as is the functionally similar epithelium of the proximal tubule of the kidney. Inflammation of the gall bladder is a fairly common problem. Without due care, a section of the gall bladder could easily be mistaken for one of small intestine. Therefore, the gall bladder provides an excellent opportunity to observe how various details can serve to distinguish organs which otherwise appear superficially similar. Since the gall bladder is really just a glandular duct, its structure is much simpler than that of intestine. The gall bladder has a wrinkled mucosa, with sections across the wrinkles resembling villi. However, in sections of gall bladder the wrinkles always appear attached to the wall and sometimes form arches. In contrast, in sections of intestine, finger-shaped villi occasionally have the appearance of separate ñislandsò, cut off from the wall. The mucosa of the gall bladder, unlike that of the intestine, has no crypts. The epithelial lining of the gall bladder consists of simple columnar cells specialized for absorption, with an apical brush border of microvilli, very similar to intestinal absorptive

cells. However, unlike intestinal epithelium, gall bladder epithelium includes only this single cell type; it has no goblet cells. Like the intestine, the gall bladder has a muscular outer wall. However, the muscular wall of the gall bladder is not organized into distinct circular and longitudinal layers, unlike the muscularis externa of the intestine. The gall bladder has no muscularis externa of the intestine. The gall bladder has no muscularis mucosae, and no distinct submucosa (David King, *et al*, 2003).

2.5 PANCREAS

The pancreas is a gland that is partly exocrine and partly endocrine, the main bulk of the gland being constituted by its exocrine part. The exocrine pancreas part of the pancreas is in the form of a serous, compound tubule alveolar gland. The pancreas is surrounded by delicate capsule. Septa extend from the capsule into the gland and divided it into lobules. The secretary elements of the exocrine pancreas are long and tubular (but they are usually described as alveoli as they rounded or oval in secretions). Their lumen is small. The lining cells appear triangular in section, and have spherical nuclei located basally. Secretions produced in the alveoli are poured into intercalated ducts (also called interlobular ducts).These ducts are invaginated deeply into the secretary elements. As a result of this invagination the ducts are not conspicuous in secretions. From the intercalated ducts the secretions pass into larger interlobular ducts. They finally pass into the duodenum through the main

pancreatic duct and accessory pancreatic duct. The endocrine part of the pancreas is in the form of numerous rounded collections of cells that are embedded within the exocrine part. These collections of cells are called the pancreatic islets, or the islets of Langerhans. The human pancreas has about one million islets. They are most numerous in the tail of the pancreas.

2.6 OESOPHAGUS

The esophagus is a strong muscular tube, about 25 cm long that delivers food to the stomach. In the relaxed state, the mucosa is deeply folded, allowing it to stretch when a bolus of food is delivered. The epithelium is stratified squamous nonkeratinized epithelium. Keratohyalin granules may be present in some of the surface cells. A closer examination of the epithelium would show that the cells in the lower to middle layers are cuboidal or polygonal, but the surface layers are always flattened, hence the designation squamous. Stratified squamous epithelium is typical of surfaces that will encounter friction. The appearance of the lamina propria is typical although lymphocytes canôt be distinguished at this magnification. Note the abundance of blood vessels, only a few of which are indicated. The boundary between the lamina propria and epithelium is distinct, but marked by irregularities where connective tissue papillae push into the epithelium. The asterisk at the left of the figure is between two such islands. Throughout, most of the esophagus, no glands are present in the lamina propria. Note; The muscularis mucosae consist of longitudinal muscle fibres and is quite

prominent while the mucosa of the esophagus has the highest simplest structure of anywhere in the alimentary canal. The appearance of the submucosa is typical as described under General Structure. Tubulo-alveolar mucous secrecting glands are scattered throughout the submocosa of the eosphagus. The muscularis externa of the of the esophagus is typical as far as the orientation of the muscle layer is concerned (inner circular, outer longitudinal). However, the muscularis externa of the upper third of the esophagus contains skeletal muscle rather than the smooth muscle, while the muscularis externa of the lower third consist entirely of smooth muscle. The general abundance of blood vessels is evident. A particular large one, filled with RBCs, is seen in the muscularis mucosae. The abundant lymphocyte appears as little purple specks. Here, some have aggregated to form a lymph nodule. No cardic-like glands are present in the lamina propria, as this section was not made from the upper-or lower lowermost parts of the esophagus. The structure of the lamina propria is best seen in the esophagus, as the LP of the stomach, small intestine and colon is filled with glands. The muscularis mucosae is quite thick in the esophagus and consists mainly of longitudal muscle fibres. The connective tissue of the submucosa of the esophagus lying between the muscularis mucosae and the part of the inner circular layer of the muscularis externa is denser than that of the lamina propria, and is considered moderately dense irregular CT. Blood vessels are also abundant. No ganglion cells or nerve bundles are seen in this section

(Meissner's plexus). Mucous glands (called esophageal glands) are scattered throughout the submucosa of the esophagus.

2.7 LIVER

Looking at a section of liver is somewhat reminiscent of looking down out of an airplane at a suburban neighborhood. One sees a very regular, almost monotonous, collection of houses in blocks demarcated by roads, with a gas station or minimart apparent at almost every intersection. In the case of the liver, the roads are connective tissue septa which convey vascular and biliary traffic, and the clusters of houses are cord-like arrangements of hepatocytes, the parenchymal cell of the liver. Sheets of connective tissue divide the liver into thousands of small units called lobules. A lobule is roughly hexagonal in shape, with portal triads at the vertices and a central vein in the middle. The lobule is the structural unit of the liver and rather easy to observe. In contrast, the hepatic acinus is more difficult to visualize, but represents a unit that is of more relevance to hepatic function because it is oriented around the afferent vascular system. The parenchymal cells of the liver are hepatocytes. These polygonal cells are joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes. The ultrastructure appearance of hepatocytes reflects their function as metabolic superstars, with abundant rough and smooth endoplasmic reticulum, and Golgi membranes. Glycogen granules and vesicles containing very low density lipoproteins are readily observed.

Hepatocytes make contact with blood in sinusoids, which are distensible vascular channels lined with highly fenestrated endothelial cells and populated with phagocytic kuffer cells. The space between endothelium and hepatocytes is called the Space of Disse which collects lymph for delivery to lymphatic capillaries. Bile originates as secretions from the basal surface of hepatocytes, which collect in channels called canaliculi. These secretions flow toward the periphery of lobules and into bile ductules and interlobular bile ducts, ultimately collecting in the hepatic duct outside the liver. The hepatic duct is continuous with the common bile duct, which delivers bile into the duodenum. In most species, bile is diverted through the cystic duct into the gall bladder. The columnar epithelium of the gall bladder is devoted largely to absorption of water and electrolytes.

2.7.1 LIVER LOBULES

The hepatic cells are aggregated into minute of regularly polyhedral blocks called liver lobules (Hepatic lobule) which in a transverse section looks hexagonal. The lobules are more distinct in pigs, rats or cat, because the connective tissue containing branches of hepatic artery, branches of hepatic portal vein, branches of bile duct and lymph vessels are collectively called the

portal canals, portal areas or portal spaces. In a transverse section, of the liver, these spaces appear triangular or frigid.

2.7.2 HEPATIC CELL

Each hepatic cell roughly polyhedral in shape and with a cell membrane. Normally each cell has a central nucleus but two or more nucleus proteins and lysosomes in addition to glycogen and fat droplets. Bile pigments may occasionally be seen wide in the cells. Though, the middle of a hepatic lobule rains a vein from the top to the bottom (central vein). Radiating from the vein towards the periphery of a tube are vertical walls of hepatic cells called hepatic plates or lamina. In cross section, the lamina looks like radiating rows of hepatic cells which were often hepatic cords surrounding the hepatic cells is a network of fire bile canaliculi.

2.7.3 HEPATIC SINUSOIDS

There are literally compressed branching sinuses in between the neighboring lamina. Very often sinusoids from one side communicate with the other. Sinusoids receive blood from branches of the hepatic portal vein and branches of hepatic artery. This blood is drained out of the lobule by a sub-lobular vein. In the lining of the hepatic sinusoids besides the endothelial cells are the phagocytic cells meant to destroy foreign particles in the blood. They are called

vein-kufferôs cell. They are irregular outline and are slightly larger than endothelia cells. Other types of cell in between the liver sinusoid are the fat storing cells which take up gold chlorine stains. They are fat droplets.

2.7.4 BILE DUCTS

In the liver, the bile canaliculi confirmed to the hepatic laminae. The end of the network of the bile canaliculi opens into ducts at the periphery of a lobule which then from larger bile duct runs along with the branches of the hepatic artery and hepatic portal vein. The smaller bile is lined by columnar cells.

2.7.5 LYMPH VESSELS

This forms a rich plexus in the Gilsonôs capsule and in connective tissue septa within the portal canals. In the sections of the liver, they cannot be clearly seen because the extremely thin wall can collapse when the tissue is fixed for sectioning.

2.8 KIDNEY

Interstitial tissue consists of reticular fibres around the nephron in the duct system and the blood vessels. Under the microscope, glomerulus and medullary rays are noticeable. The medullary rays will be seen as radically directed thin striated from middle of the cortex to the middle of the medulla. Actually, these are bundles of collecting tubules under high power proximal and distal

convoluted tubules are distinguishable from the collecting tubule. Proximal tubule has cuboidal cells with brush borders (Microvilli) and their lumen is small. The distal tubules also have cub cell but the brush border is absent and their lumen is larger. The collecting tubules have cuboidal cells with distinct outlines and darkly stained nuclei. The venal capsule is composed of the glomerulus and Bowmanôs capsule.

2.8.1 MEDULLA (Renal tubules)

Renal tubules functions like exaggerated striated ducts, modifying the filtrate by reabsorbing everything that is not waste. Renal tubules have wiggly portions, called convoluted tubules, straight segments, called loops of Henle, and collecting ducts. Different aspects of filtrate reabsorption are localized in these different segments. The initial segment of the tubule is the proximal convoluted tubule. It is called proximal because it is nearest to the starting point (the renal corpuscle) and convoluted because it twists about (in contrast to the straight segments of tubule which form the loop of Henle). This segment of the renal tubule restores much of the filtrate to the blood in the peritubular capillaries, by actively pumping small molecules out of the tubule lumen into the interstitial space. (Water then follows the concentration gradient.) The length of a proximal convoluted tubule tends to be several times greater than that of a distal convoluted tubule, so sections of proximal tubules are much more common than those of distal tubules in a typical histological slide of renal cortex. The

epithelium of the initial descending segment of the loop of Henle is similar to that of the proximal convoluted tubule, and is sometimes called the pars recta of the proximal tubule (in contrast to the convoluted pars convoluta). The proximal convoluted tubule is lined by a simple cuboidal epithelium whose cells have several characteristic features. The apical end of each cell has a brush border of microvilli. This provides an increased surface area to accommodate the membrane channels that are responsible for absorbing into the cell small molecules from the filtrate in the tubular lumen. The brush border is seldom plainly visible in routine histological preparations, but proximal tubule cells tend to have indistinct apical ends (in contrast to the more definite apical border of cells comprising distal tubules and collecting ducts). The cells have a high proportion of mitochondria in their cytoplasm, to provide the energy for pumping ions and molecules against their concentration gradient. The abundance of mitochondria makes the cells rather intensely acidophilic. The plasma membranes of adjacent proximal tubule cells are extensively interdigitated. This increases the basal membrane surface area available for pumping molecules out the basal end of each cell. As a consequence of such interdigitated cell membranes, boundaries between adjacent proximal tubule cells are inconspicuous (i.e., in section the epithelium looks like a continuous band of cytoplasm with nuclei appearing at irregular intervals). The distal convoluted tubule is lined by a simple cuboidal epithelium whose cells have several characteristic features. Unlike the proximal convoluted tubule, the apical

end of each distal tubule cell does not have a brush border, although there may be scattered microvilli. Because most of the ñheavy liftingò has already been done in the proximal tubule, distal tubule cells are not so highly specialized. The apical ends of distal tubule cells tend to be more distinct than those of proximal tubule cells, conferring the usual appearance of a larger, clearer lumen in each distal tubule. Distal tubule cells have a high proportion of mitochondria in their cytoplasm, to provide the energy for pumping ions and molecules against their concentration gradient. However, distal tubule cells are less extremely specialized than those of the proximal tubules. They have fewer mitochondria and therefore a lesser degree of acidophilia (David, 2011).

2.8.2 CORTEX (Renal corpuscles)

In the kidney, each renal corpuscle is essentially just a highly modified secretory acinus. Bowmanôs capsule is the outer, epithelial wall of the corpuscle. Bowmanôs space, also called ñurinary spaceò, is the space lying within Bowmanôs capsule. The glomerulus is the conspicuous ñlittle ballò which occupies most of the corpuscle, comprising several distinct elements. Glomerular capillaries have an endothelium that is fenestrated (full of holes). Podocytes are epithelial cells covering the glomerular capillaries. Immediately adjacent to each glomerular capillary, in between the podocytes and the capillary endothelium, is the filtration membrane (not labelled on this diagram). Mesangium is a supporting tissue consisting of mesangial cells and matrix. Bowmanôs capsule is the outer epithelium which encloses Bowmanôs space.

This epithelium is simple squamous, becoming cuboidal at the proximal tubule. Although Bowmanôs capsule is rather obviously a simple squamous epithelium, it is less apparent that the glomerulus is also closely enveloped by epithelium. The peculiar structure of podocytes obscures the fact that they are indeed epithelial cells. Thus Bowmanôs space is entirely lined by epithelium. The outer, ñparietalò epithelium of the renal corpuscle is Bowmanôs capsule. The inner, ñvisceralò epithelium is comprised of podocytes. One way to appreciate the essential epithelial construction of renal corpuscles is by examining fetal development. Each renal corpuscle is roughly spherical and has two ñpolesò at opposite ends. The vascular pole receives the afferent and efferent arterioles, which serve the glomerular capillaries. The urinary pole is the location of the proximal tubule, the outflow for the glomerular filtrate. Associated with the vascular pole is the juxtaglomerular omp1ex. Note: Capillary endothelial cells line the fenestrated glomerular capillaries while Podocytes stand upon pedicels (ñlittle feetò) on the outer side of the glomerular capillaries. Mesangial cells are concentrated between capillaries at the vascular pole of the corpuscle (David, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Standard histological methods and materials were put in use as described by (Bancroft and Marilyn, 2002).

3.1 ANIMAL CARE ETHICS

The animal study was carried out in compliance with policies outlined in the óGuide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). For the research to commence, approval was obtained from the animal care unit; Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Upon approval, the rats were obtained and housed in plastic cages with saw dust as beddings to acclimatize, in the animal house of the department of Medical Biochemistry, University of Nigeria, Enugu campus for 2 weeks, under standard condition of temperature (25 ± 5ºC) and a light/dark periodicity of 12:12 hrs. Enough food (Standard pellet (Guinea feed) and commercially produced table water was provided).

3.2 ANIMAL GROUPING.

For this study, 30 (thirty) in-bred male and female white rats of about 2-4 months old and 100-200 g body weight were randomly selected into Six (6)

groups of 5 rats per cage and would be labeled as cages 1, 2, 3 and 4 with cages 5 and 6 serving as control.

3.3 EMPIRICAL MEASUREMENT

The method described by (Ajiboso, *et al*, 2007) was used to determine body weight of experimental rats. Individual rat were monitored for daily gain in body weight using digital electronic balance (Gilbertini, Italy). Gain in weight was calculated from the relationship given below: Daily gain in weight= Final day Weight ð Initial day Weight, while the average mean weight of 198.86 g was noted.

3.3.1 PHYSICAL MEASUREMENT

Behavioral signs of acute toxicity as observed in experimental rats was noted; such as: diarrhea, watery stool, hair loss, stretching, reduced activities, dullness, restlessness, paw licking and salivation.

3.4 EXPERIMENTAL PLANT

2 kg of fresh leaves of *V. album* where purchased from plantation in Umunede, Ika Local Government Area of Delta State, Nigeria and were identified as *V. album* at Botany Department of University of Nigeria Nsukka, Enugu State, Nigeria.

3.4.1 PHYTOCHEMICAL SCREENING

The solvent extract of *V. album* was subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituents using the method described by (Borokini and Omotayo *et al*, 2012; Anokwuru, *et al*, 2011 and Egwaikhide, *et al,* 2007).

TEST FOR FLAVONOIDS

5 ml of diluted ammonia solution was added to a portion of the aqueous filtrate of plant extract, followed by addition of concentrated sulphuric acid . Colour changes were observered to draw inference.

TEST FOR TRITERPENES

5 ml of chloroform was added to 0.5 g of extract in a dry sterile test tube and was shaken for 5 minutes. It was filtered and the filtrates was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Golden yellow colouration was observed and used to draw inference being indicative of the presence of triterpenes.

TEST FOR SAPONINS

Using a sterile test tube, 30 ml of tap water was added to 1 g of the powdered leaf sample. The mixture was vigorously shaken and heated concurrently. The heated mixture was observed for the formation of froth/foams which was taken as preliminary evidence to draw inference.

TEST FOR ALKALOIDS

0.5 g of extract was stirred with 3 ml of 1 % aqueous hydrocholic acid on a steam bath and filtered, 1 ml of the filtrate was treated with few drops of Picric acid solution. Colour changes/precipitate were taken as preliminary evidence to draw inference.

TEST FOR ANTHRAQUINONES

5 ml of choloroform was added to 0.5 g of extract in a dry sterile test tube and was shaken for 5 minutes. It was filtered and the filtrate shaken with equal volume of 100% ammonia solution. Pink to violent colouration in the ammonical layer (lower layer) was observed and was used to draw inference being indicative of the presence of free anthraquinones.

TEST FOR PHENOLS

0.5 g of extract was treated with 5 % ferric chloride. Deep blue or black colouration was observed and was used to draw inference being indicative of the presence of phenols.

TEST FOR PROTEINS

0.5 g extract was treated with 3 ml of concentrated nitric acid. Yellow colouration was observed and used to draw inference being indicative of the presence of protein.

TEST FOR PHYTOSTEROLS

0.5 g extract was added to 5 ml of choloform and shaken for 5 minutes. It was filtered and the filtrate was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Brown ring at the junction was observed and used to draw inference being indicative of the presence of phytosterols.

The phytochemical present in the sample from the leaves of *V. album* were juxtaposed with their enthno-medicinal significance and from these; several suggestions were deduced on the secondary metabolite responsible for the pharmacological actions of the plant (Borokini and Omotayo, 2012).

Table 1.0: Qualitative phytochemical analysis of *V.album* extract

S/	Secondary	Positive indicators	Absent/

N	metabolites		Present
01	Alkaloids	Orange precipitate	Absent
02	Saponins	Present of froths/foams	Present
03	Flavonoids	Yellow precipitate	Present
04	Phytosteriols	Brown ring	Present
05	Anthraquinones	Pink violet or red colouration at lower layer (ammonical layer)	Absent
06	Phenols	Deep blue/black colouration	Present
07	Proteins	Yellow colouration	Present
08	Triterpenes	Golden yellow colouration	Present

The plant revealed present of the following secondary metabolites saponins, flavonoids, phytosteriods,proteins,triterpenes and phenolic compounds.

3.5 PREPARATION OF PLANT EXTRACT

Fresh leaves of *Viscum album* from the host plant (citrus) were collected. The leaves were first washed free of sand and debris. It was thoroughly washed with tap water and allowed to air dried under shade for 1 week. The dried leaves were pulverized into uniform powder using a house hold blender. The extract was obtained by measuring 50g of the uniform powder to 1000 ml of distilled water. It was heated in hot air oven at 95^{o}C for 15 minutes, using normal household gas cooker. The heated, mixture was transferred to a shaker (Model

GFL No 3017) while shaking sustained continously for 6 hrs. The extract was filtered using Whatman No.1 filter paper. It was refrigerated ready for use.

3.5.1 ACUTE TOXICITY TEST LD_{50}

Acute toxicity of the extract was obtained using modified Lorkeôs method (1983). Forty male and female rats were used for the study. They were allowed a week for adaptation. The rats were grouped into two. The first group of twenty (20) rats received one of the following doses; 50, 100, 150, and 200mg/kg body weight of extract orally. The rats were observed for signs of toxicity which include salivation, stretching, weakness, reduced activities, sleep and death in first four (4hrs) and subsequently daily for 7days. The second group of twenty (20) rats received one of the following doses; 1000, 2000, 3000 and 4000 mg/kg body weight of extract orally. There were assessed for mortality within the first four (4hrs) and thereafter daily for 7 days. The oral median lethal dose was calculated using the formula; $L.D_{50}$ = ã Minimum toxic dose x Maximum tolerated dose.

3.6 DESIGN AND CONDUCT OF EXPERIMENT

The thirty (30) rats were divided into 6 groups of 5 rats each. Cage 1, 2, 3, 4 were treated with extract of mistletoe orally in order of 100, 150, 200 and 300 mg/kg body weight respectively of the extract while cages 5 and 6 served as the

control groups without experiment with regards to the $L.D_{50}$ of 547.72 mg/kg body weight. At the end of the feeding period, the animals were sacrificed; organs of interest were excised, washed with normal saline and preserved in 10% neutral buffered formalin awaiting tissue processing.

3.6.1 REAGENTS AND CHEMICALS

Scottôs water, Distilled water, Chloroform, Haematoxylin dye, Eosin stain, 1% acid - alcohol, Xylene, Ethanol, Distrene Plasticizer, 10% neutral buffered formalin and Normal saline (All reagents were distilled prior to use).

3.6.2 EQUIPMENT AND APPARATUS

Analytical Weighing Balance, Stainless Steel Cages, Dissecting Materials: Dissecting Board, Dissecting Set, Cotton Wool. Glassware (Pyrex): Measuring Cylinder, Conical Flask, Cover slip, Slides, Universal Containers, 5ml syringes. Tissue Processing Materials: Automatic Tissue processor, Leuckhart Moulds, Rotary Microtome, Water Bath (Gallenkamp), Hot Plate, Staining rack, Forceps and Swift binocular microscope (Olympus England).

3.7 LOCATION AND DURATION OF STUDY

This study was conducted in the department of Medical Laboratory Science, Histopathology sub-departmental Laboratory, Faculty of Sciences and technology, College of Medicine, University of Nigeria, Enugu campus. Animal

acclimatization, test administration, Grossing, Tissue processing, microtomy, Staining and microscopic examination of histological sections lasted for 2 months (June 1st to August 2nd).

3.8 STATISTICAL ANALYSIS

Data were analyzed and result presented as Mean Standard Error and analyzed using one way Anova and Duncan post hoc test and significance was determined at probability less than 0.05.

3.9 PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

- Dewax and hydrate section.
- Stain section in Coleôs Hematoxylin for l0mins.
- Wash section thoroughly in running tap water. Differentiate section in 1% acid alcohol briefly.
- Blue in Scotôs water for 5minutes.
- Counter stain section in eosin for 3minutes.
- Wash section in running tap water until excess eosin has been removed.
- Dehydrate section in ascending grades of alcohol (70%, 90% and absolute).
- Clear section in xylene, mount in DPX (Avwioro, 2000; Baker, *et al*, 2001).

CHAPTER FOUR

4.0 RESULT

4.1 MICROSCOPY AND PHOTOMICROGRAPHY

The sections were examined using Swift binocular microscope with an in built light system and white films with an Olympus photomicroscope.

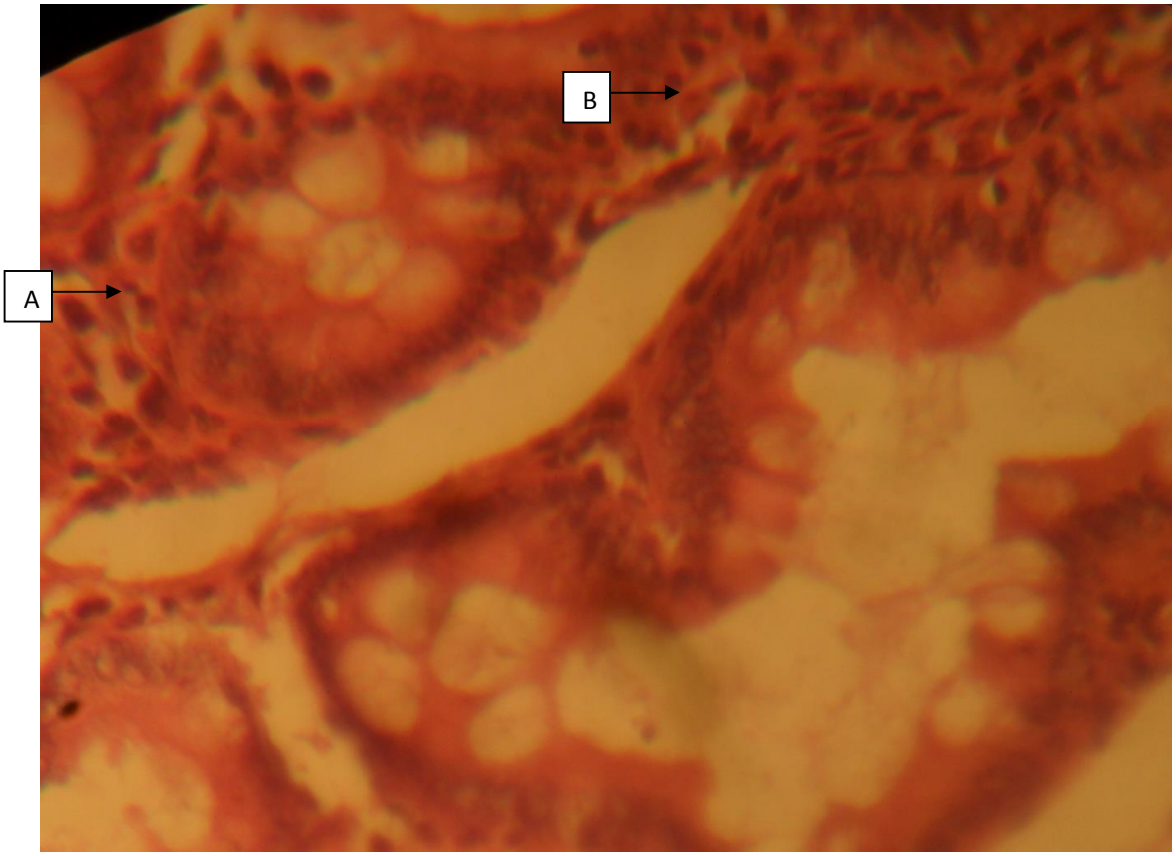

Fig 1: Rat duodenum treated with 100 mg/kg of *V. album* for 28 days showing mild mucosal infiltrates of inflammatory cells A and congestion B (H&E x 40)

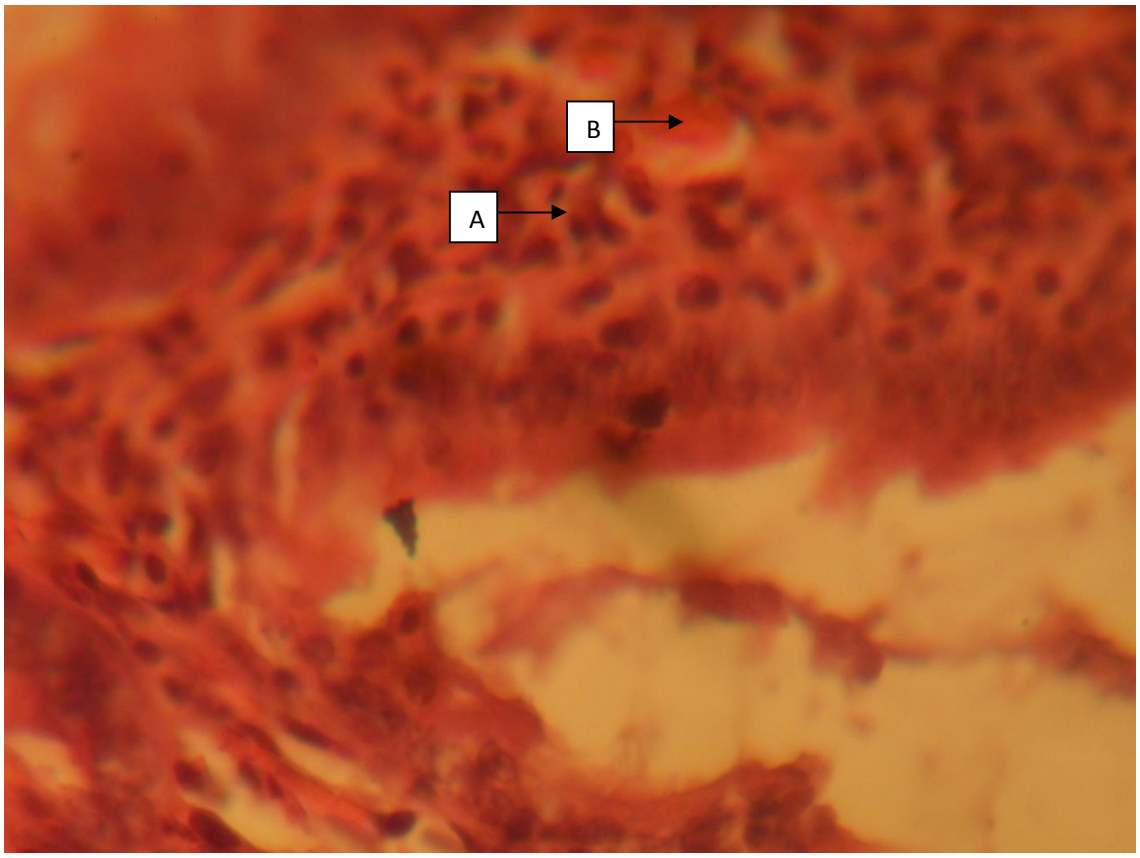

Fig 2: Rat duodenum treated with 150 mg/kg of *V. album* for 28 days showing moderate mucosal infiltrates of inflammatory cells A and congestion B (H&E x 40)

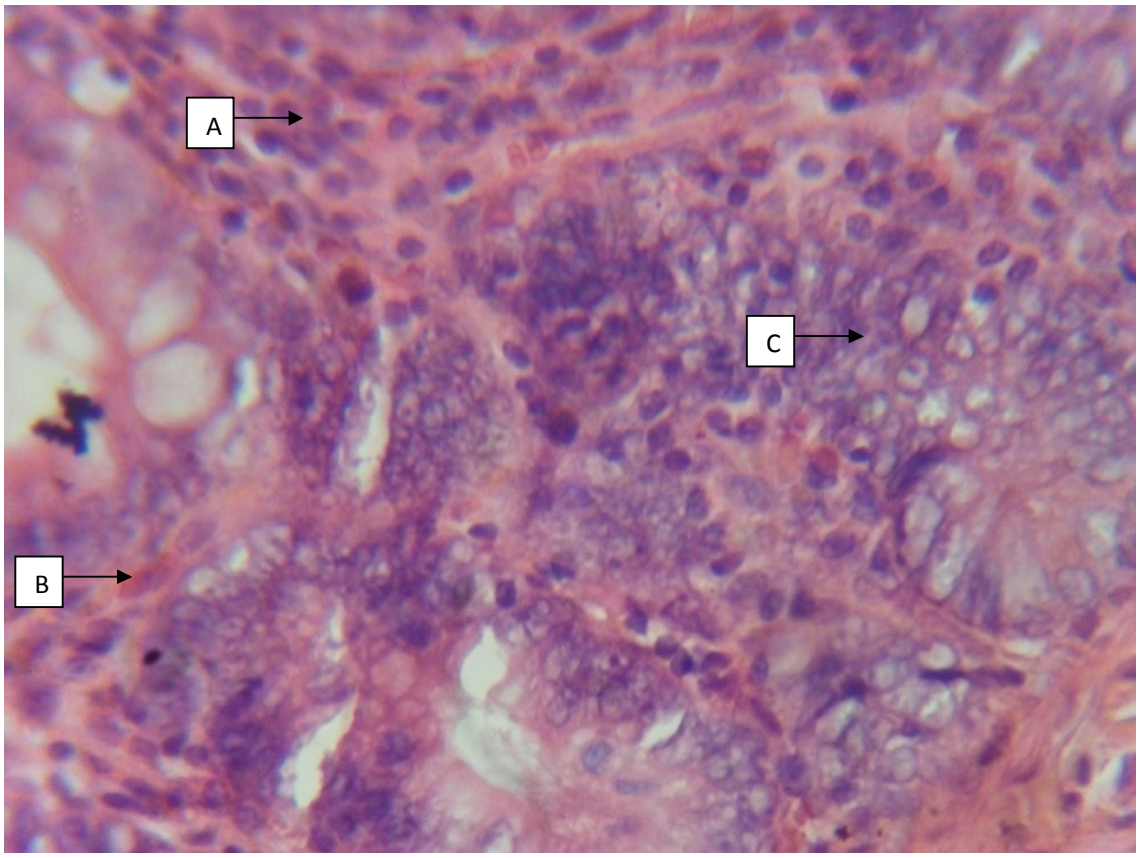

Fig 3: Rat duodenum treated with 200 mg/kg of *V. album* for 28 days showing moderate mucosal infiltrates of inflammatory cells A mild congestion B and mild glandular hyperplasia C (H&E x 40)

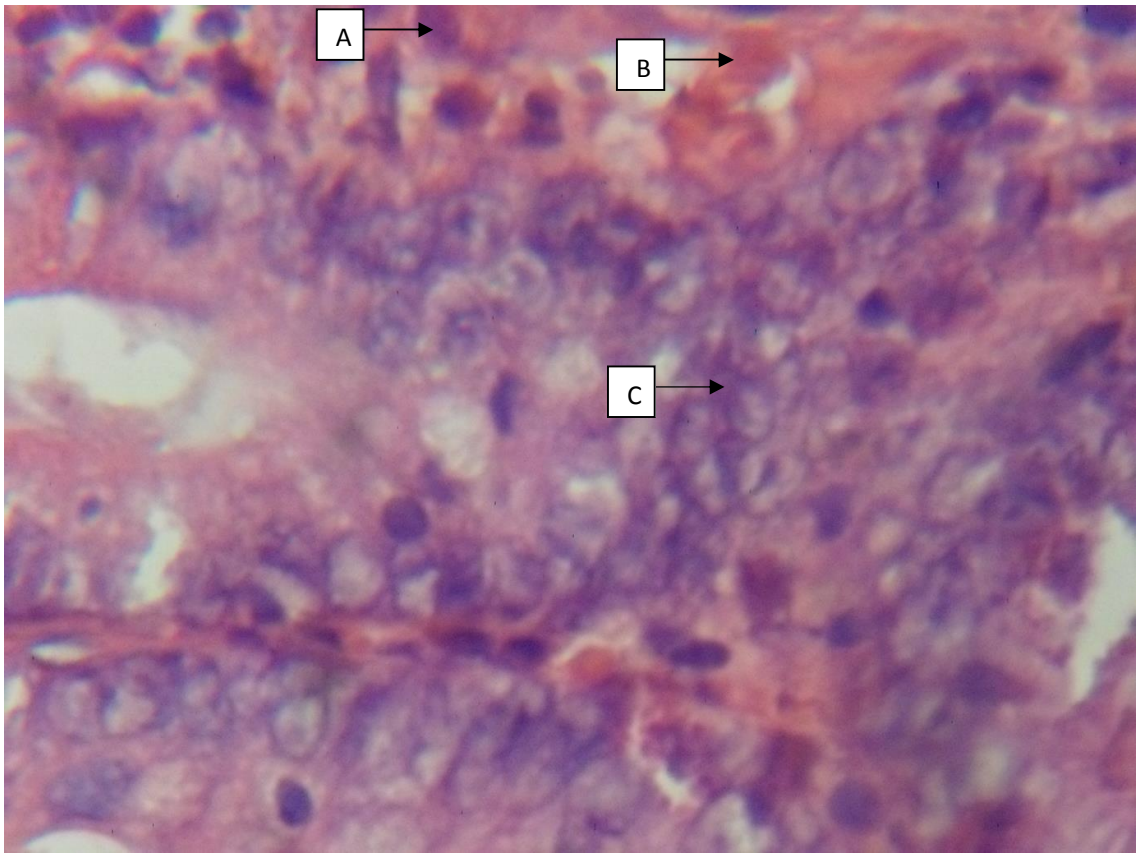

Fig 4: Rat duodenum treated with 300 mg/kg of *V. album* for 28 days showing moderate mucosal infiltrates of inflammatory cells A mild congestion B and mild glandular hyperplasia C (H&E x 40)

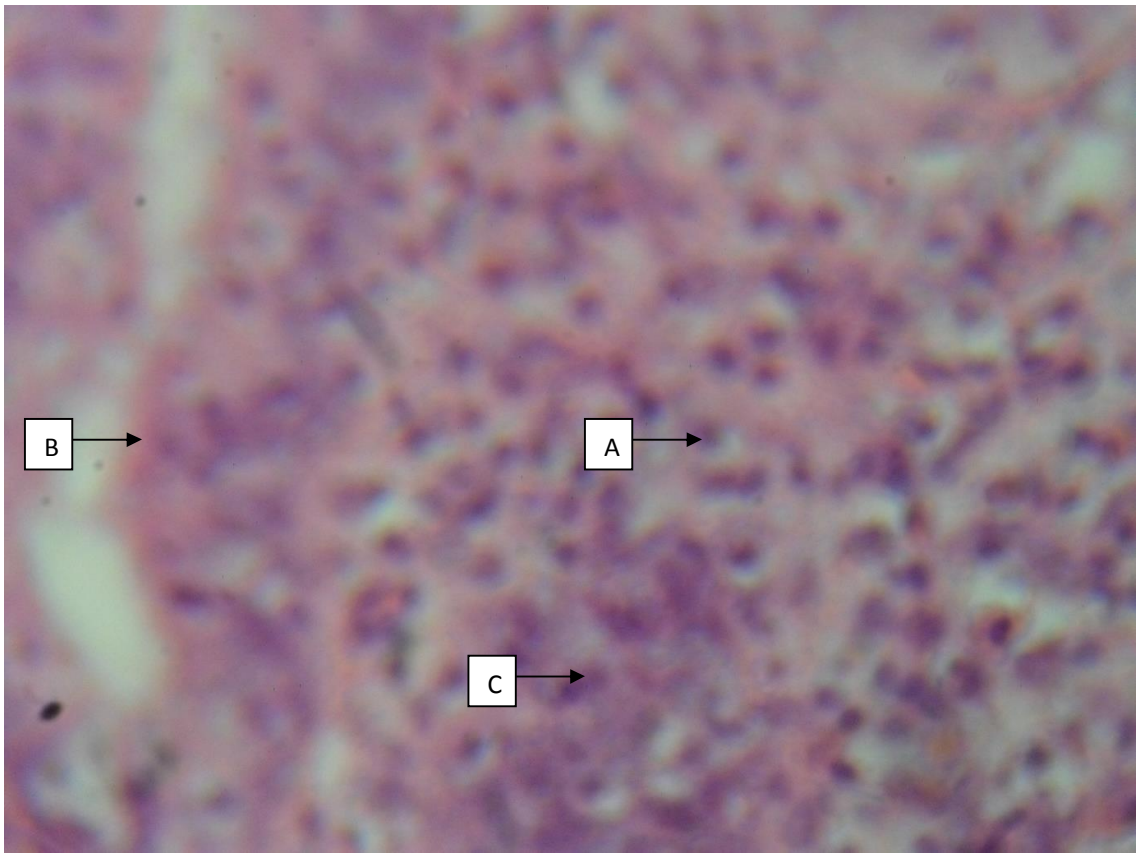

Fig 5: Rat gall bladder treated with 100 mg/kg of *V. album* for 28 days showing mild mucosal infiltrates of inflammatory cells A mild congestion B and mild glandular hyperplasia C (H&E x 40)

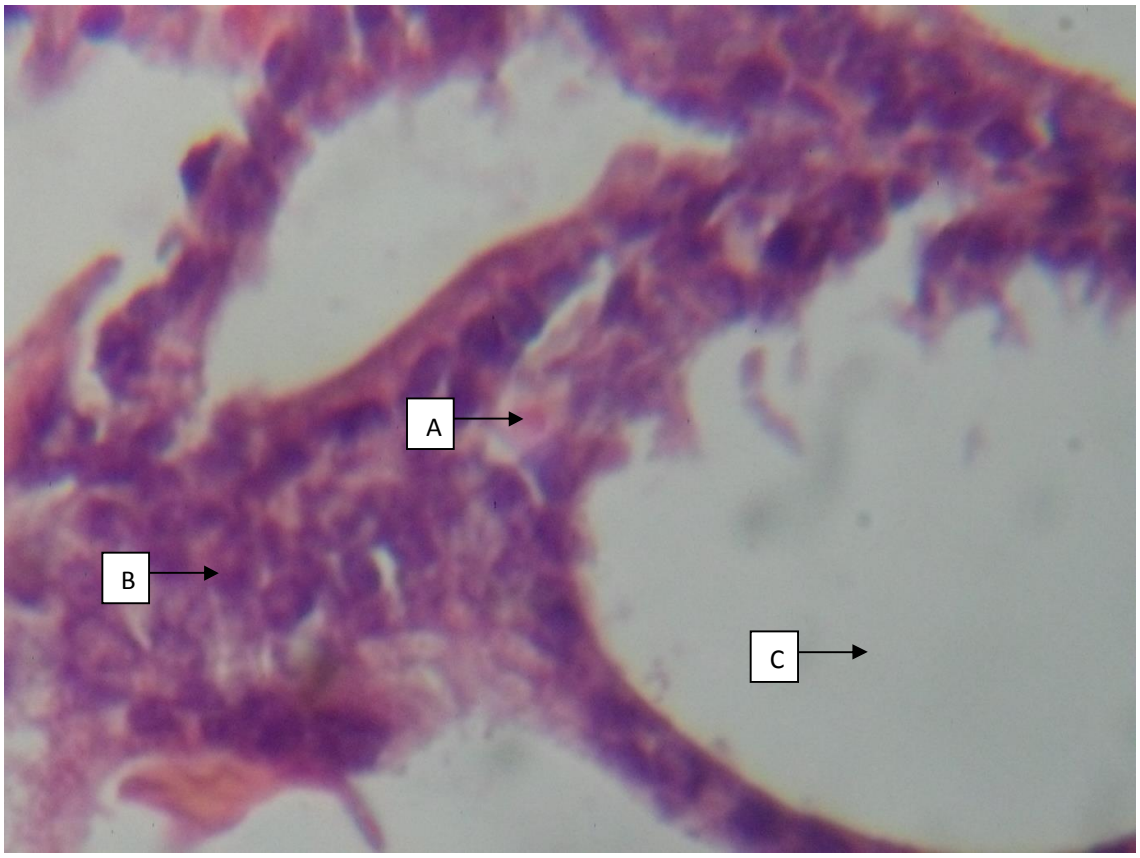

Fig 6: Rat gall bladder treated with 150 mg/kg of *V. album* for 28 days showing mild congestion A mild glandular hyperplasia B and cystic glandular dilation C (H&E x 40)

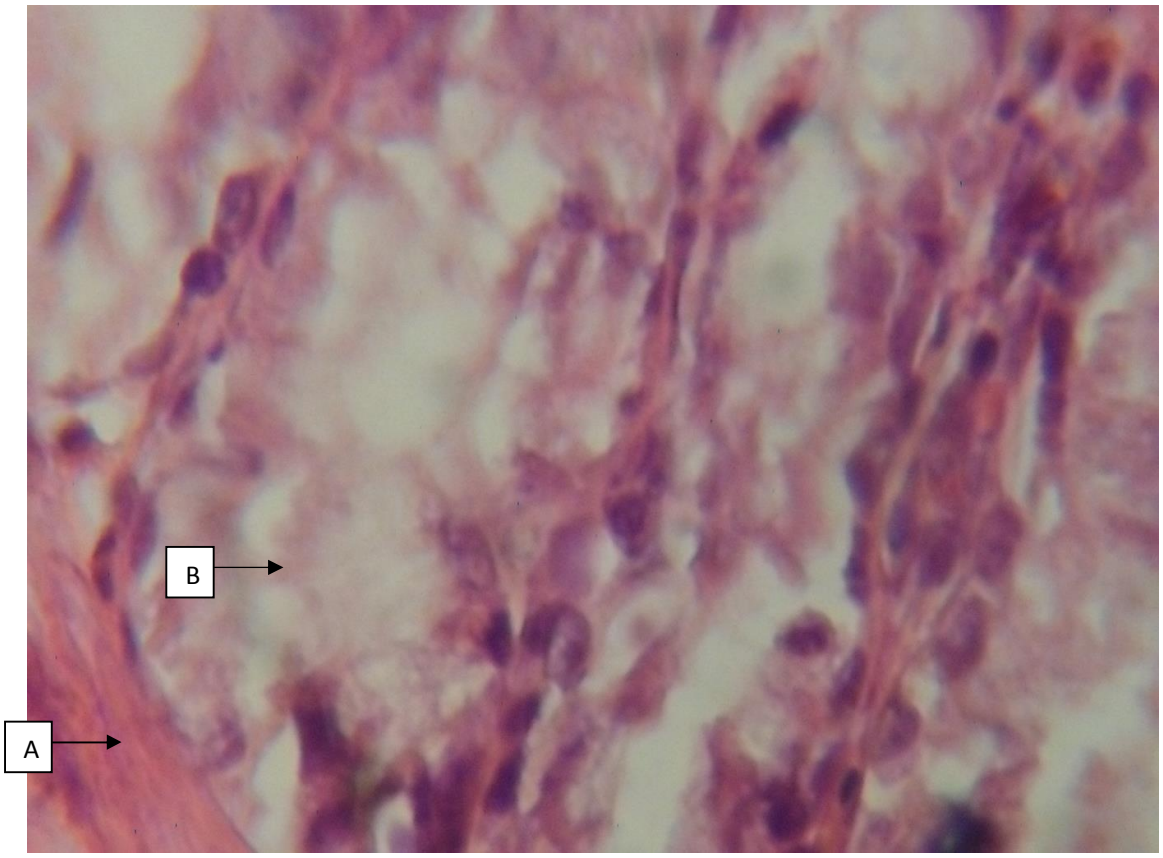

Fig 7: Rat gall bladder treated with 200 mg/kg of *V. album* for 28 days showing mild congestion A and mild glandular hyperplasia B (H&E x 40)

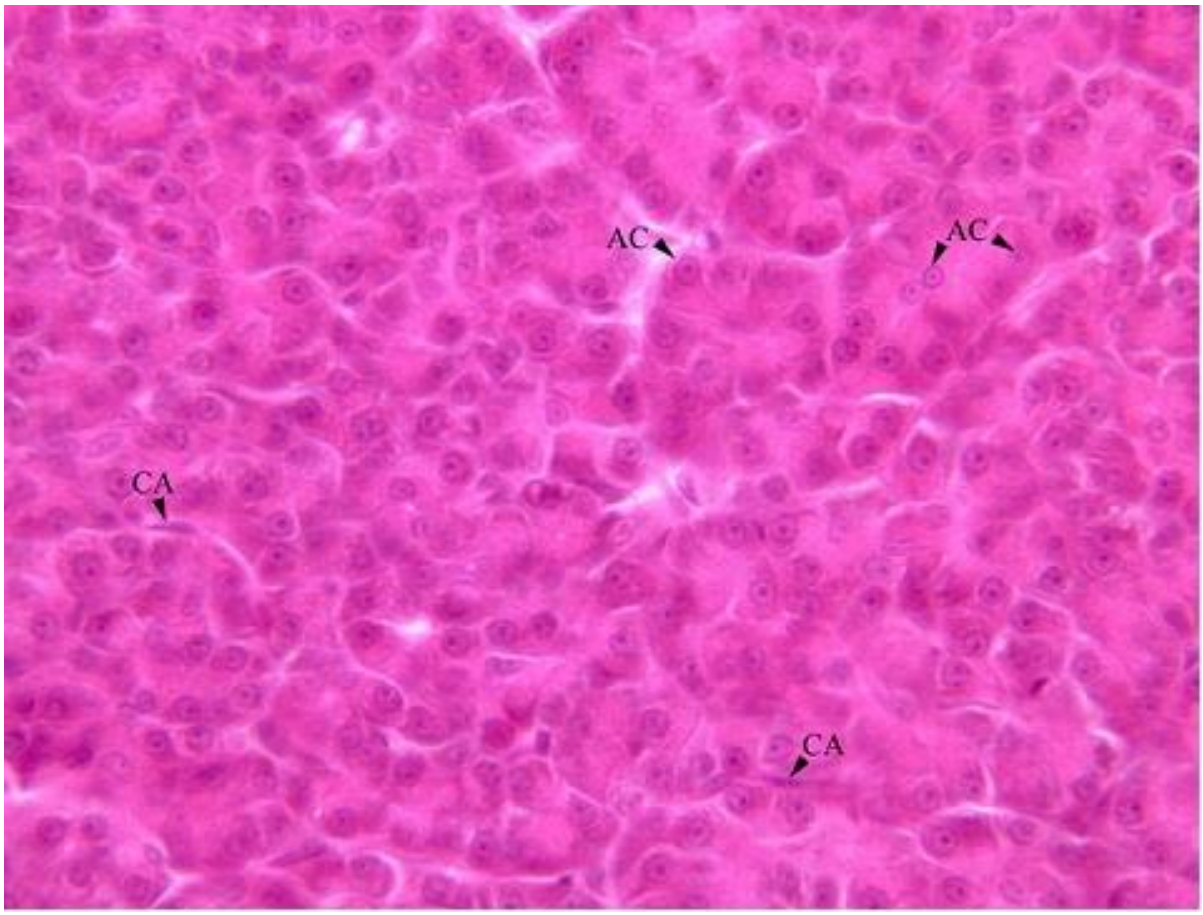

Fig 8: Control Rat pancreas showing Acinar AC and Centroacinar cells.

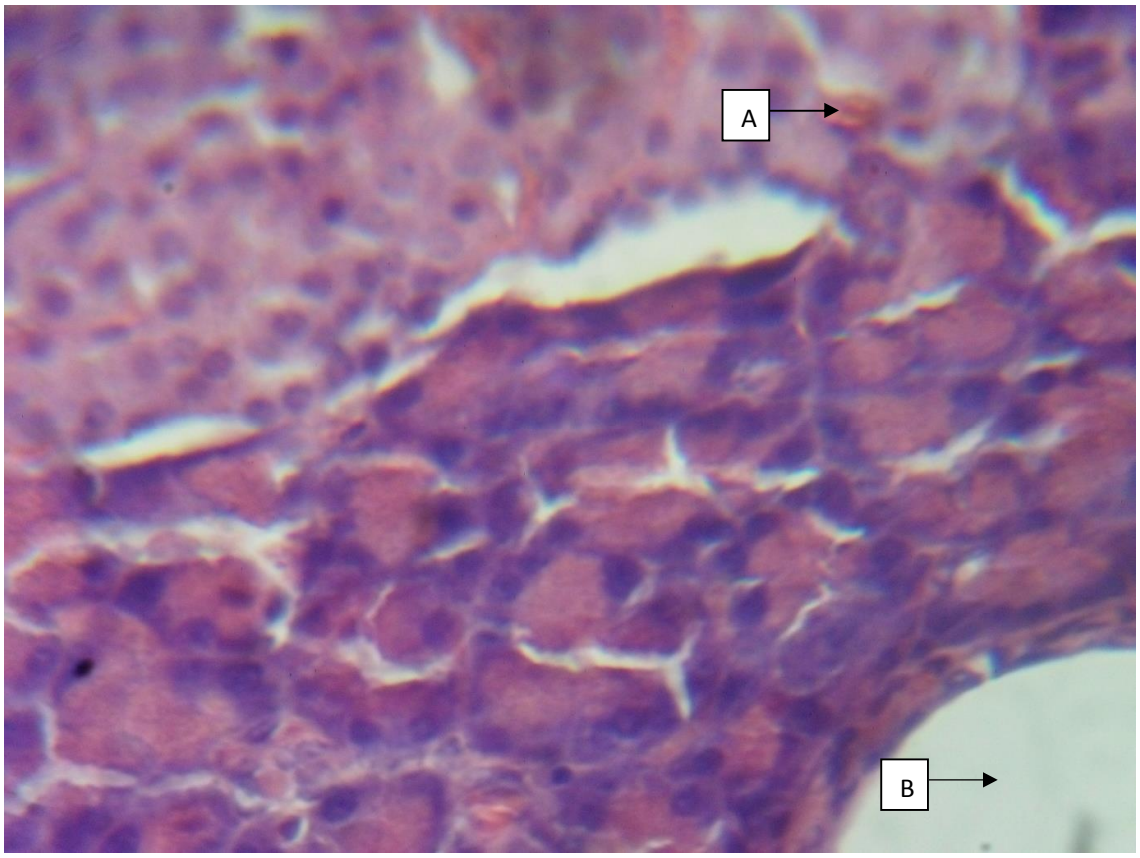

Fig 9: Rat pancreas treated with 100 mg/kg of *V. album* for 28 days showing mild islet congestion A and mild ductal dilation B (H&E x 40)

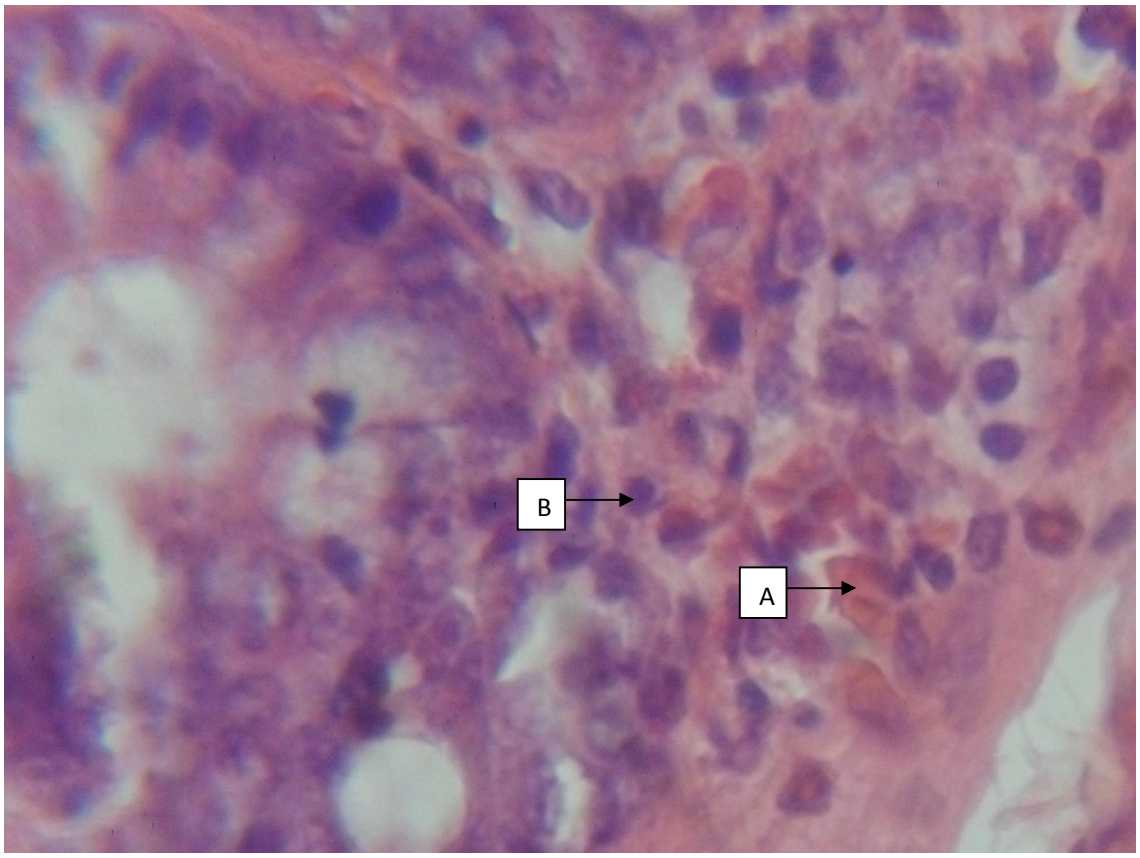

Fig 10: Rat oesophagus treated with 150 mg/kg of *V. album* for 28 days showing mild mucosal congestion A and mild infiltrates of inflammatory cells B (H&E x 40)

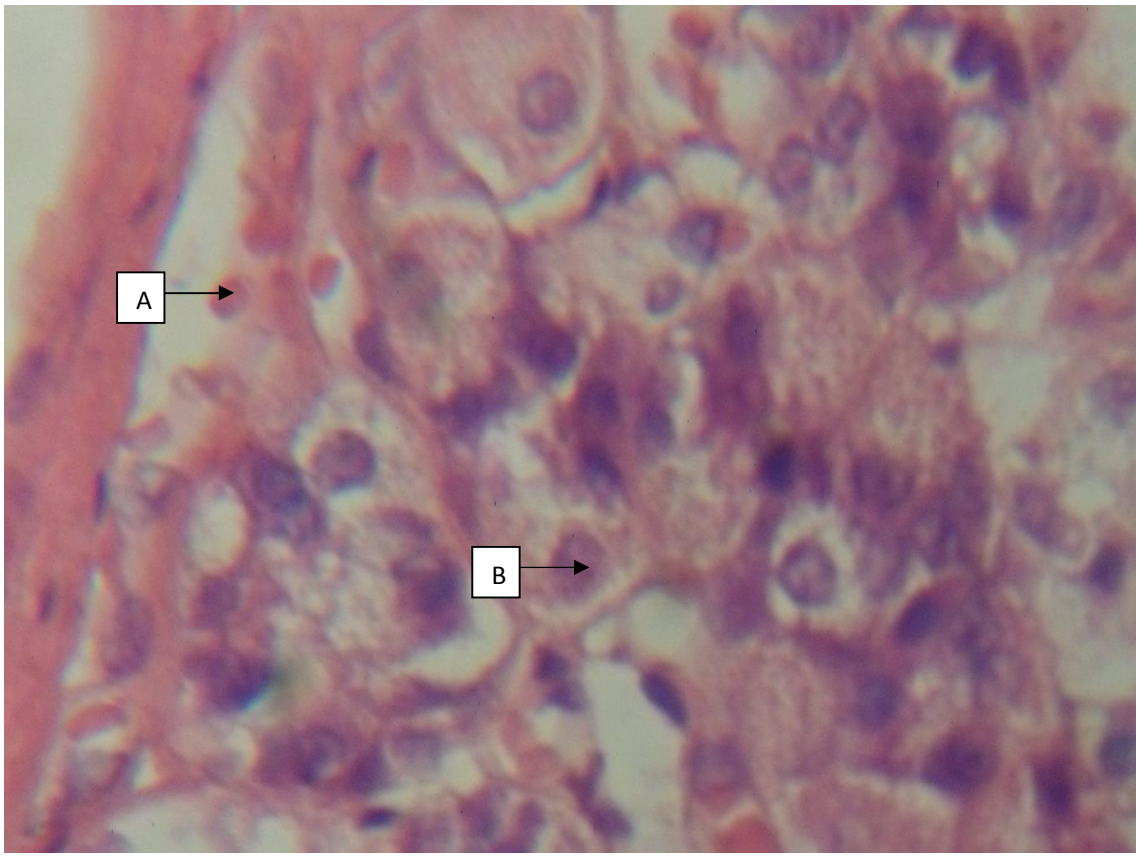

Fig 11: Rat oesophagus treated with 300 mg/kg of *V. album* for 28 days showing mild mucosal congestion A and mild glandular hyperplasia B (H&E x 40)

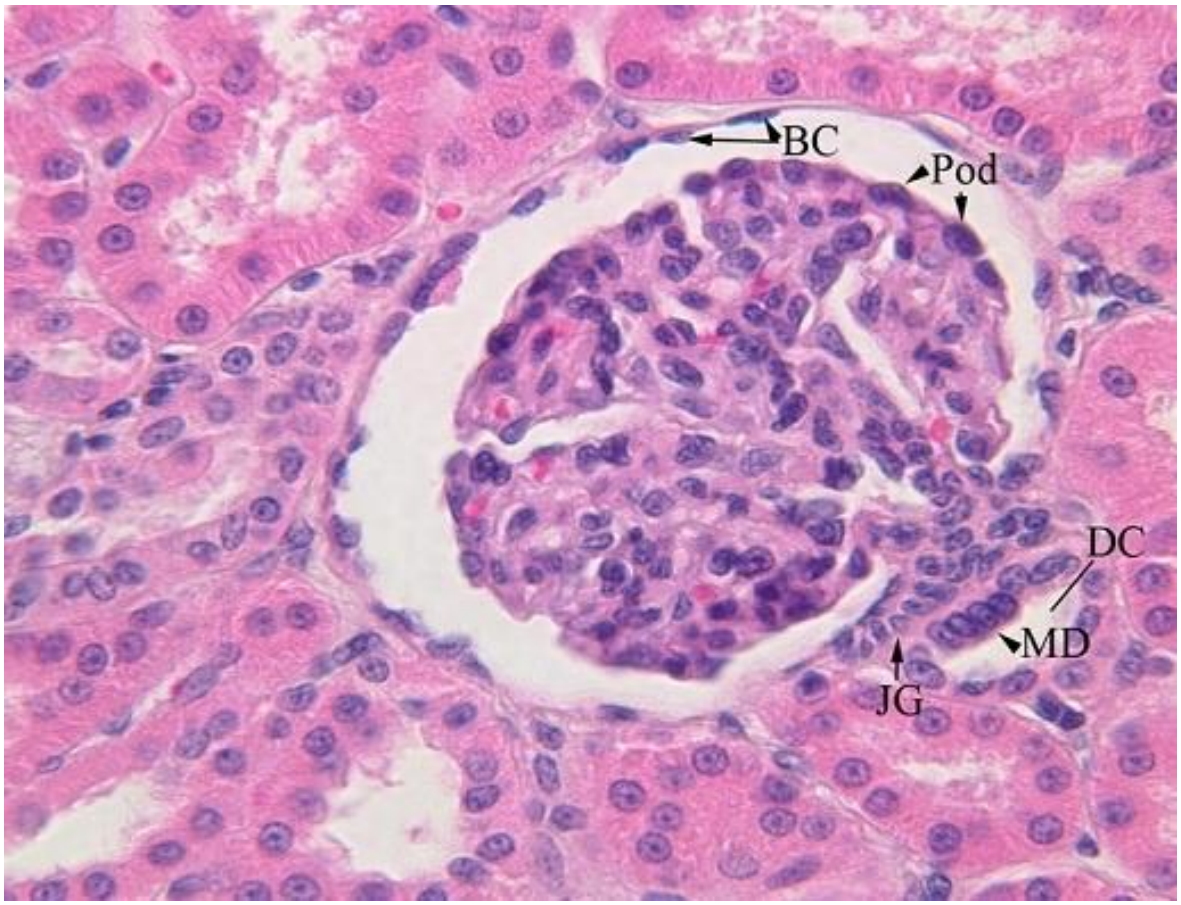

Fig 12: Control Rat kidney composed of cortical glomeruli, Bowmanôs capsule BC, Podocyte Pod, Macula densa, Juxtaglomelar cells JG, Distal convoluted tubule DC (H&Ex40).

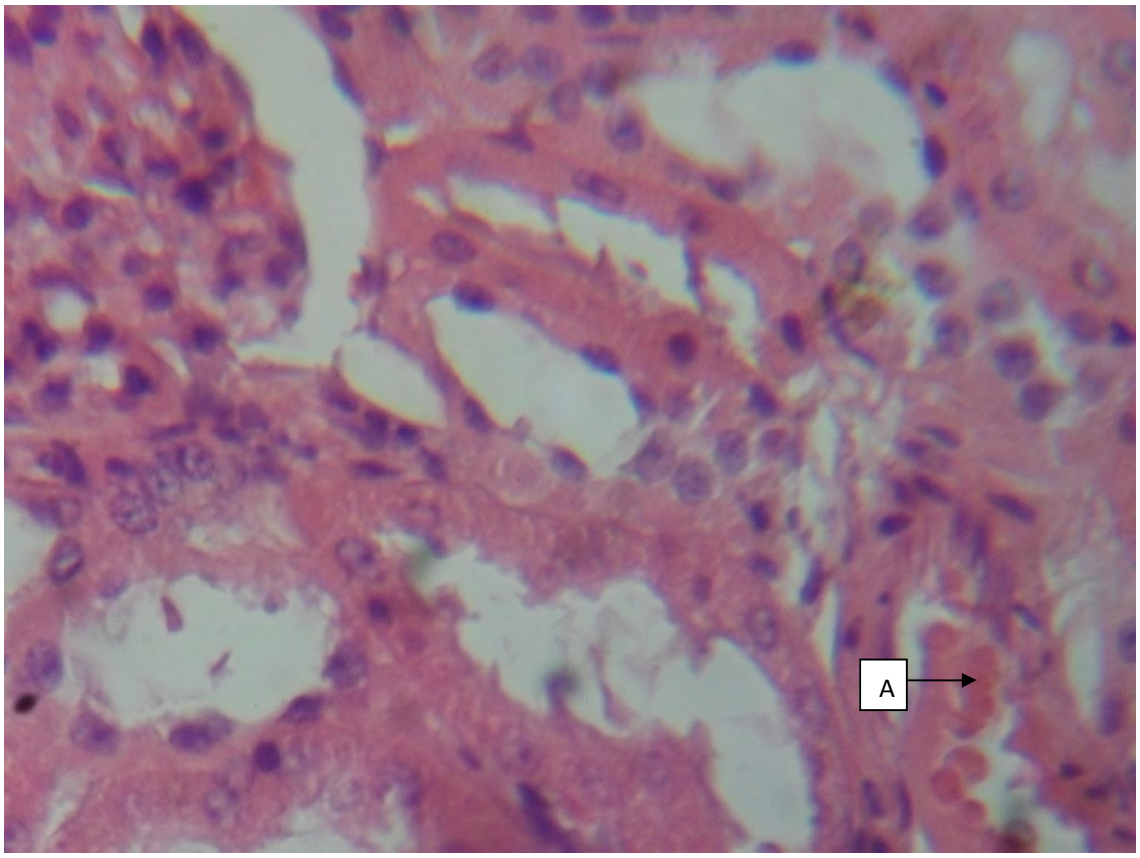

Fig 13: Rat kidney treated with 100 mg/kg of *V. album* for 28 days showing mild interstitial congestion A (H&E x 40)

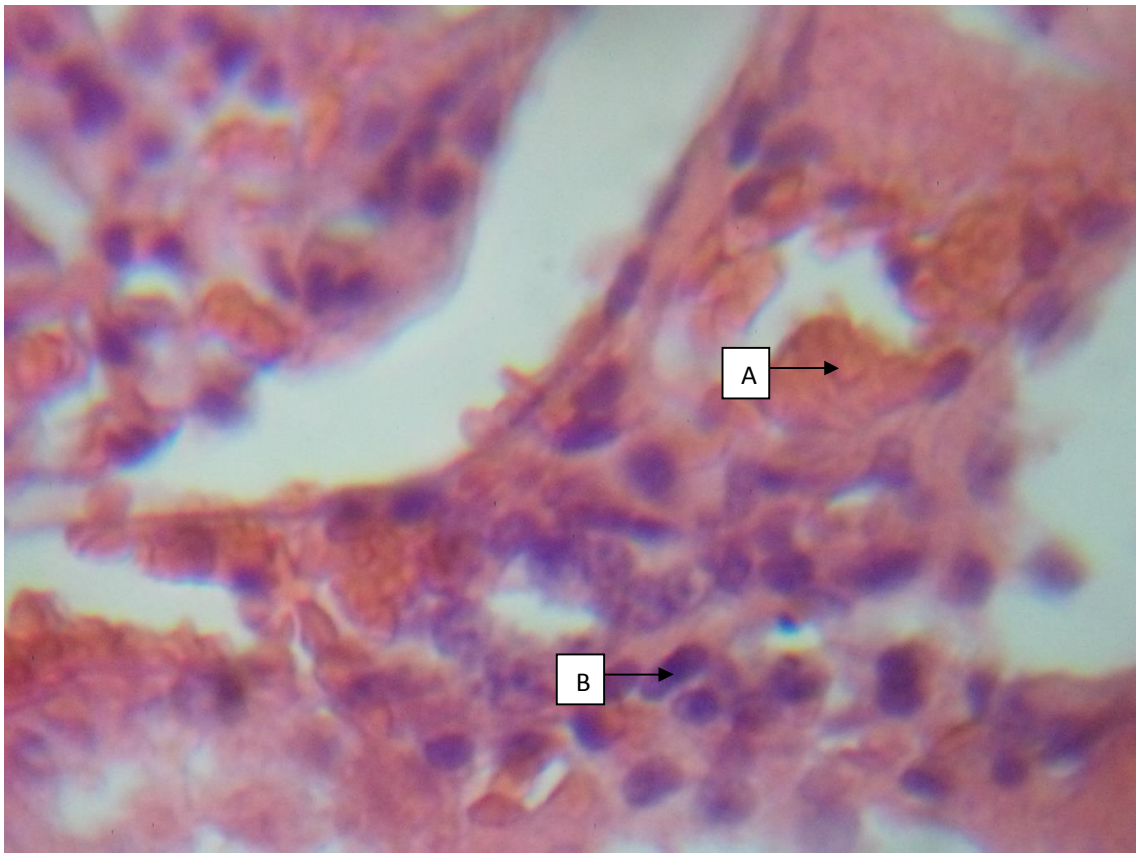

Fig 14: Rat kidney treated with 150 mg/kg of *V. album* for 28 days showing mild interstitial congestion A and mild interstitial infiltrates of inflammatory cells B (H&E x 40)

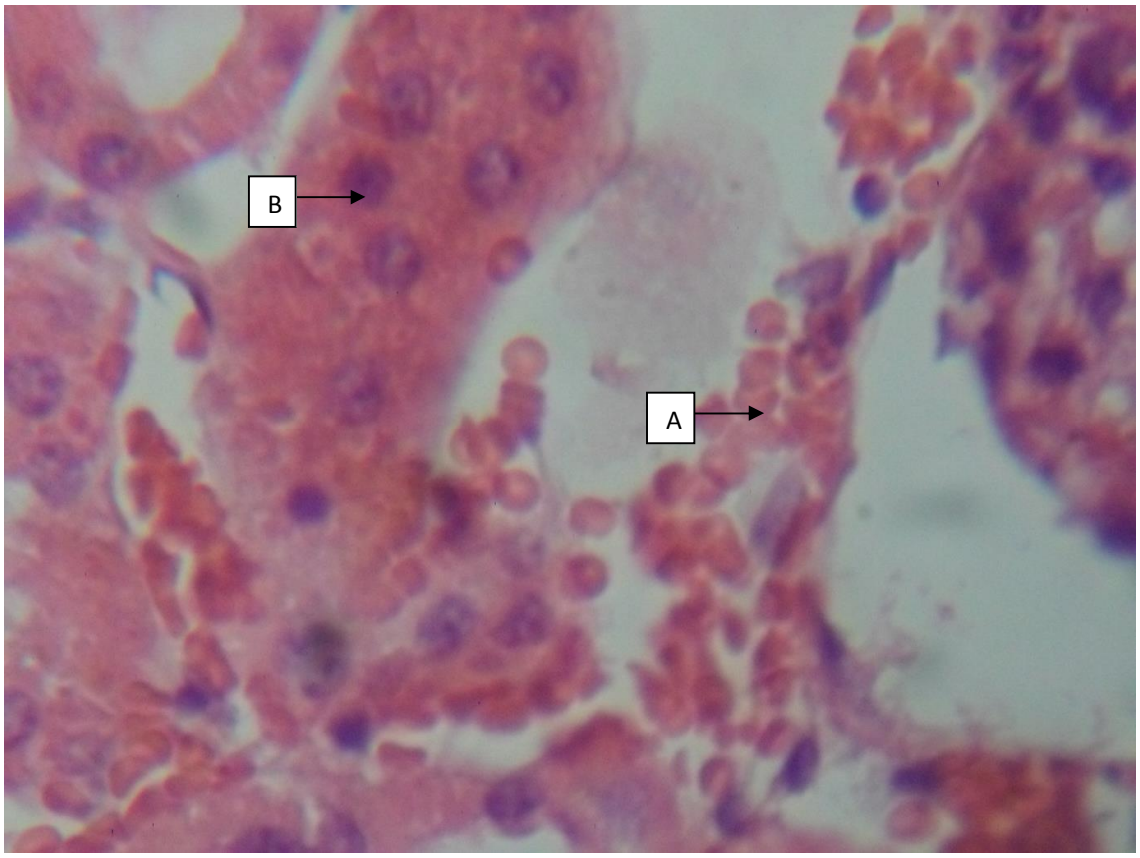

Fig 15: Rat kidney treated with 200 mg/kg of *V. album* for 28 days showing mild interstitial congestion A and mild tubular necrosis B (H&E x 40)

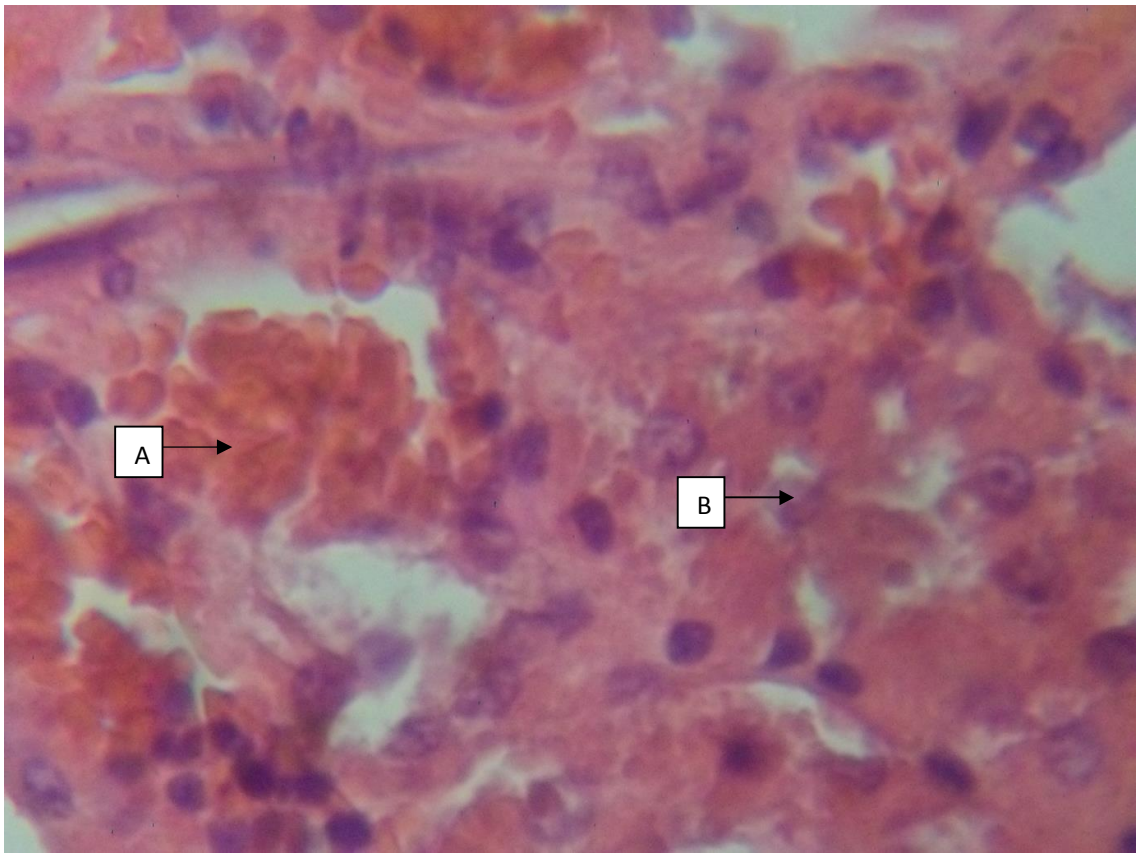

Fig 16: Rat kidney treated with 300 mg/kg of *V. album* for 28 days showing mild interstitial congestion A and mild tubular necrosis B (H&E x 40)

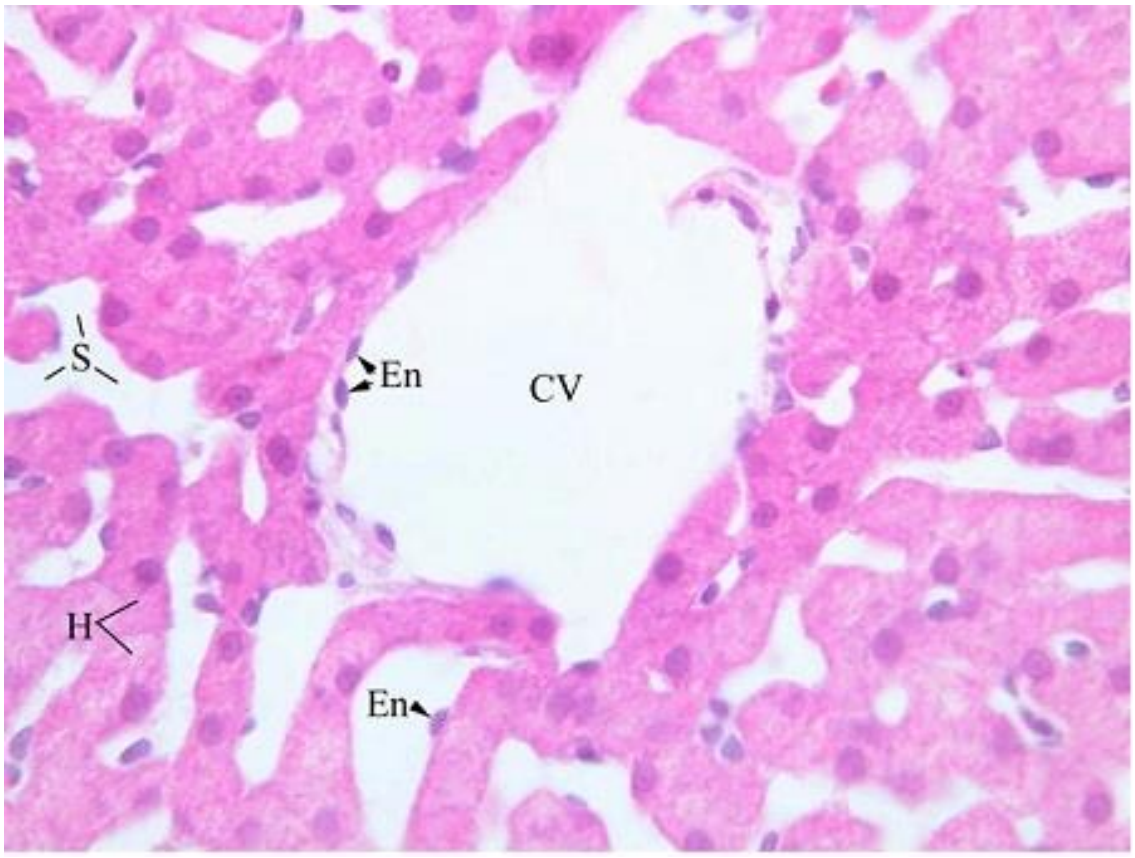

Fig 17: Control: Rat liver showing Central vein CV, Enthothelial cells En, Sinusoids S and Hepatocytes H (H&E x 40)

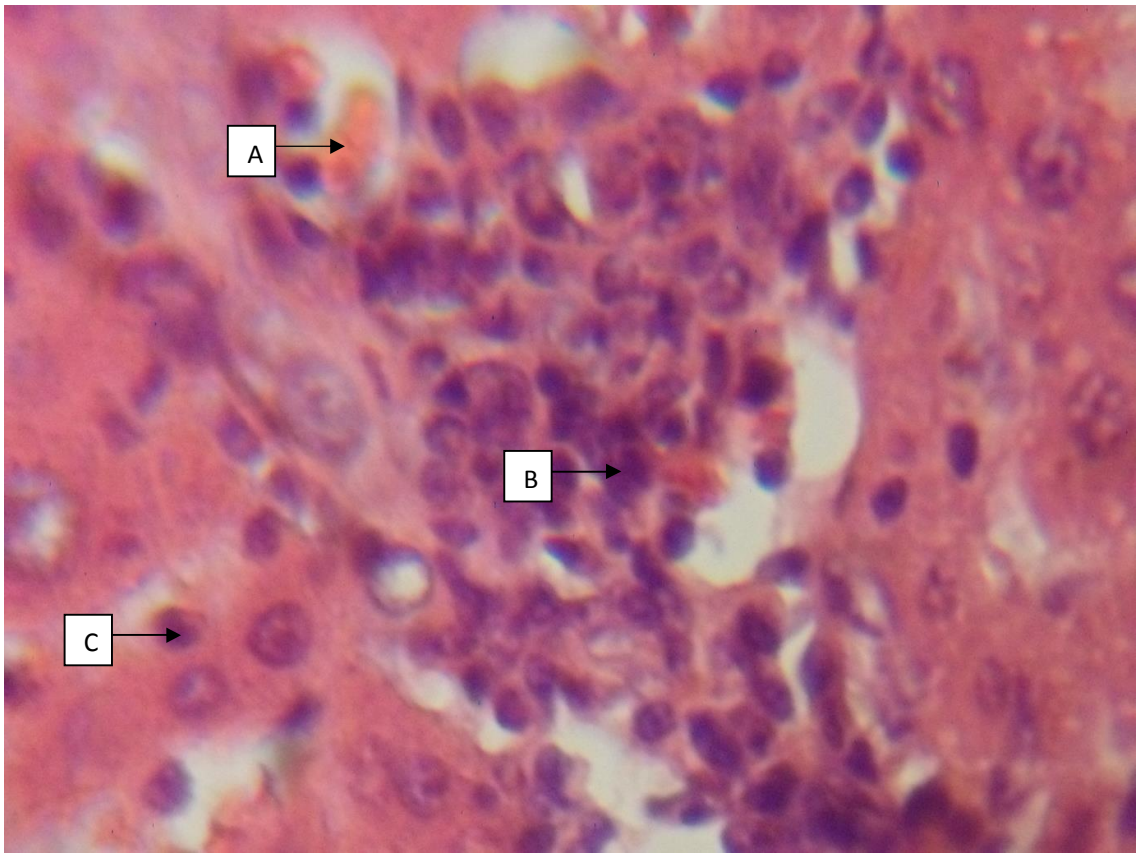

Fig 18: Rat liver treated with 100 mg/kg of *V. album* for 28 days showing mild vascular congestion A mild periportal lymphocytosis B and mild kuffer cells C (H&E x 40)

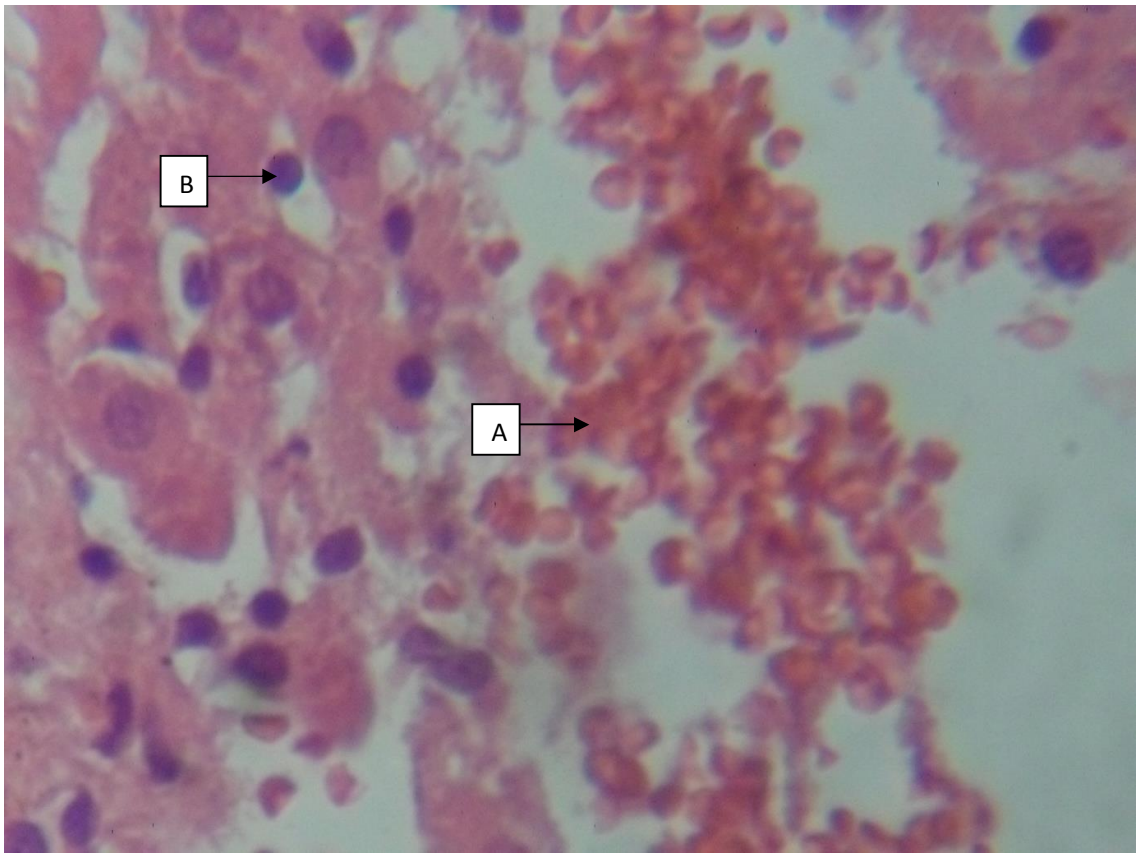

Fig 19: Rat liver treated with 150 mg/kg of *V. album* for 28 days showing mild hepatocyte haemorrhagic necrosis A and mild kuffer cells B (H&E x 40)

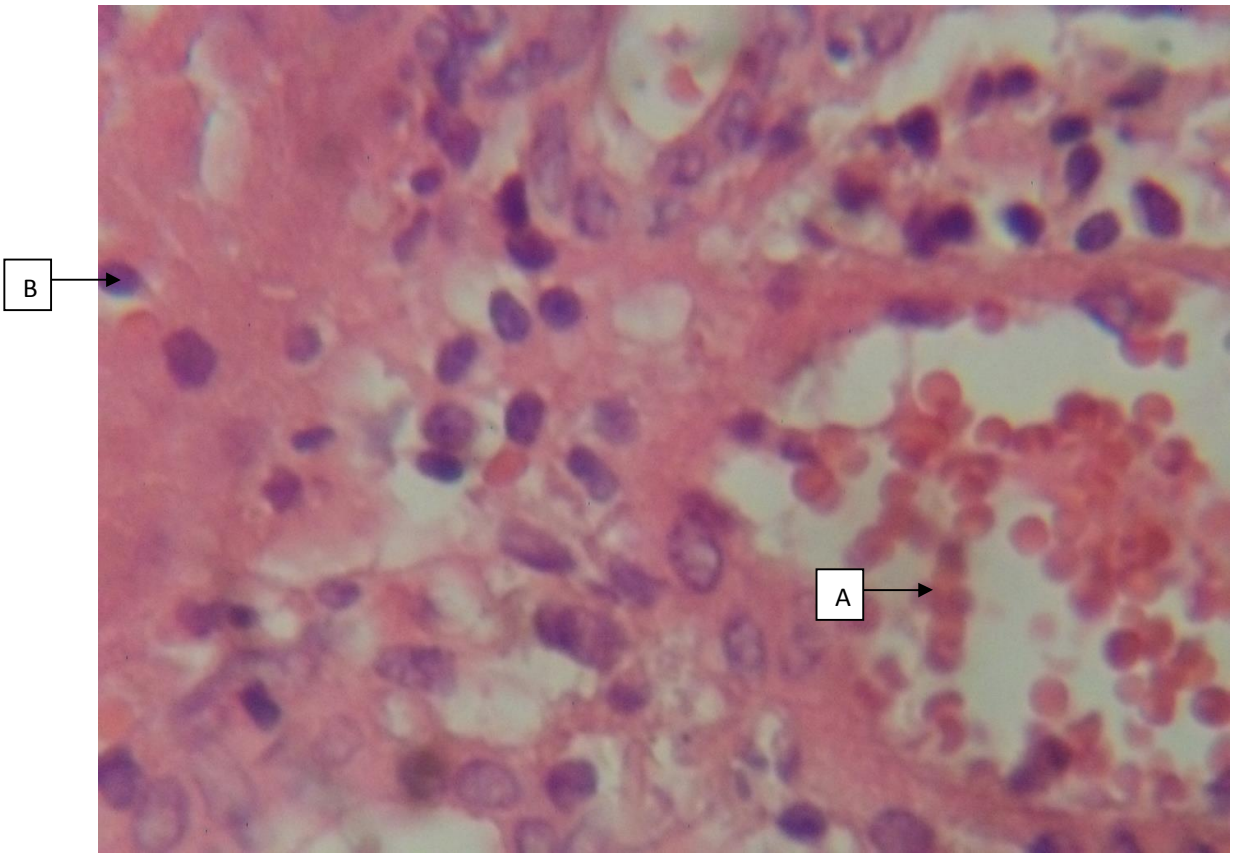

Fig 20: Rat liver treated with 200 mg/kg of *V. album* for 28 days showing mild hepatocyte haemorrhagic necrosis A and mild kuffer cells B (H&E x 40)

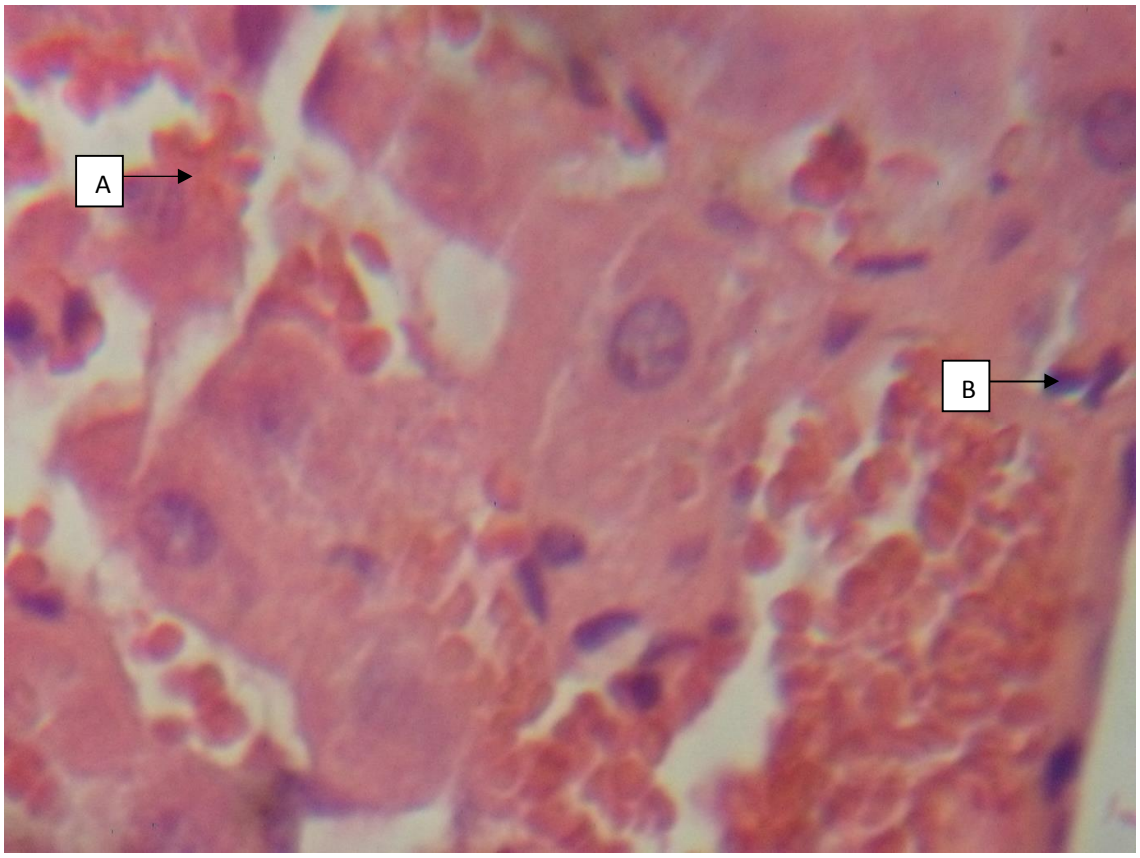

Fig 21: Rat liver treated with 300 mg/kg of *V. album* for 28 days showing mild hepatocyte haemorrhagic necrosis A and mild kuffer cells B (H&E x 40)

4.2 HISTOLOGICAL STUDY

4.3 OESOPHAGUS

In the oesophagus, administration of *V. album* at 100 mg/kg body weight induced mild mucosal inflammation however with increase dose, there was in addition, mild glandular hyperphasia.

4.4 GALL BLADDER

In the gall bladder, administraton of *V. album* at100 mg/kg body weight induced mild mucosal glandular hyperplasia and inflammation. With increase dose the same changes were observe.

4.5 DUODENUM

In the duodenum, the changes included ranged from mild mucosal inflammation at 100 mg/kg body weight to mild mucosal epithelial cell and glandular hyperplasia. Again there was no significant different as the dose increase.

4.6 PANCREAS

In the pancreas, administration of *V. album* at 100 mg/kg body weight induced mild vascular congestion and interlobular ductal dilation.

4.7 LIVER

In the liver, administration of *V. album* at 100 mg/kg body weight showed mild periportal inflammation and kuffer cell. Increase dose of the extract (150, 200

and 300 mg/kg body weight) respectively induced mild hepatocyte haemorrhagic necrosis.

4.8 KIDNEY

In the kidney, administration of *V. album* at 100 mg/kg induced mild interstitial congestion. However, with increase dose, it induced mild interstitial congestion as well as mild tubular necrosis.

4.9 DISCUSSION

This present study was designed to study the effect which the consumption *Viscum album* leaves may have on the esophagus, duodenum, pancreas, gall bladder, liver and kidney. The study also explored the possibility of using the extracts as safe alternative to orthodox medications. Infact, herbal medicine still remains the first line of medication amongst a vast majority of Africans (Barbara and Peter, 1992). The mistletoe (*V. album*) leaf extract is said to possess antidiabetic (Obatomi, *et al*, 1994) immunodulatory (Solar, *et al,* 1998), bacteriostatic (Fulder, 1998) and therapeutic values for many other ailments.

According to World Health Organization (WHO) more than 80% of the worldôs population relies on traditional medicine for their primary health care, majority of which use plants or the active principles (Gupta, *et al*, 2005). In recent past, attention has been directed towards medicinal plant research to substantiate the claims of cure made by traditional healers thus providing scientific basis for

their efficacy (Olukoya, *et al*, 1993. Results of preliminary oral acute testing performed on the albino rat revealed on the aqueous extracts of V. album had a median lethal dose of 547.72 mg/kg body weight, which is just a little above the highest administrated dose level of 300mg /kg body weight. This not just the extract administered in the study is relatively unsafe for consumption and toxic to the organs of the albino rat.

Histopathological findings in the organs studied revealed normal features for sections of the oesophagus, duodenum, pancreas, gall bladder, liver and kidney of the control group which received no extract. The sections of the oesophagus, of the treated group with administration of *V. album* at 100 mg/kg body weight induced mild mucosal inflammation however with increase dose, there was in addition, mild glandular hyperplasia. In the duodenum administration, of *V. album* leaf extract at 100mg /kg body weight ranged from mild mucosal inflammation to mild mucosal epithelial cell and glandular hyperplasia. There was no significant different as the dose increase. In the pancreas, administration of *V. album* leaf exact at 100mg /kg body weight induced mild vascular congestion and interlobular ductal dilation. In the gall bladder, administration of the extract at 100mg /kg body weight induced mild mucosal glandular hyperplasia and inflammation. With increase dose the same changes were observe. In the liver, administration, of *V. album* leaf extract at 100mg /kg body weight revealed mild periportal inflammation and kuffer cell. Increase dose of the extract at (150, 200 and 300 mg/kg) respectively induced

mild hepatocyte haemorhagic necrosis. The liver is considered as an accessory organ to the digestive system because of its crucial role in digestion, metabolism of macromolecules and storage of glycogen. It also plays an important role in the detoxification of substance including drugs by inactivating them prior to excretion. The liver in most case also attempts to inactivate poisons and toxins in which case the hepatocytes can become damaged. In the kidney, administration of *V. album* leaf extract at 100 mg/kg body weight induced mild interstitial congestion. However, with increase dose, it induced mild interstitial congestion and tubular necrosis.

Aqueous extracts of the European mistletoe (*V. album*) have been widely used for decades as alternative treatment and adjuvant cancer therapy, particularly in ceremony, Austria and Switzerland. The European mistletoe (*V. album*) extracts are used in an adjuvant cancer therapy because of their immunostimulatory and simultaneously cytotoxic properties. There effects are usually more evidence for the whole extract than for purified mistletoe lectins and viscotoxins alone (Eggenschwiler, *et al*, 2007). The therapeutic benefit of *V. album* in diverse pathologies is attributed to the method of preparation, the proportion of various bioactive compound present within the extracts and also to the host trees. Several lines of evidence have released that *Viscum album* preparations extert an anti ï tumoral effect because the ability to activate mild kuffer cells and to inhibit cell proliferation, providing a strong basis for their application as complementary therapy in cancer. *Viscum album* (mistletoe) is a

member of the Loranthacae family. It has been reported to have a number of medicinal properties including the ability to lower blood pressure, slow the heart beat, stimulate the immune system, relax spasms and extert sedative, diuretic and anti-cancers (Brown, 1995).

The photochemical screening of *V. album* from this study showed the presence of flavonoids, saponins, proteins, phytosterols, triterpenes and phenolic compounds. Results of photochemical analysis are in agreement with the earlier studies where the primary chemical constituents have been found to vary according to the host plant but typically induce glycoprotein, polypeptides (viscotoxin), flavoniods, flavonol agylcones (methyl ethers of quercentin and kaemforal), lectins such as *V. album* agylcitionin 1, ll and lll, triterpenes, saponins, caffeic acid, lignans, choline derivatives related to acetycholine, vitamin c, histamine, resins, thionins cardionolids and phenolic compounds (Edlund, *et al* 2000; Wollenweber, *et al* 2000; Lyu, *et al* 2000). However the photochemical screening of Tapinanthus dodoneifolius (DC) Danser called ñKauchiò in Hause a species of African mistletoe, showed the presence of anthraquinones, saponins, tannins (Deani and Sadiq 2000). This presence of various bioactive compounds justifies the use of the leaves by traditional practioners.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

Considering all results obtained in this study, it can be inferred that the consumption of *Viscum album* damages the oesophagus, duodenum, pancreas, gall bladder, liver and kidney both in moderate and high dose. *Viscum album* leave extract was not well tolerated. Prolonged, constant consumption of plant may lead to destruction of the tissues and loss of renal mass in the liver and kidney respectively. Further studies of this species is needed with the aim of identifying other host plant with no serious life threatening and undesirable effects.

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