# TOXICOLOGICAL ASSESSMENT OF THE METHANOL LEAF EXTRACT OF *SECURINEGA VIROSA*(ROXB EX. WILLD) IN WISTAR RATS

**BY**

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**NIGERIA**

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**FEBRUARY, 2016**

## DECLARATION

I declare that the work in this dissertation entitled **'Toxicological assessment of methanol leaf extract of *Securinega virosa* (Roxb Ex. Willd)** in Wistar rats' has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Salihu Tanimu TANKO ……. …….

Name Signature Date

## CERTIFICATION

This dissertation entitled 'TOXICOLOGICAL ASSESSMENT OF METHANOL LEAF EXTRACT OF *SECURINEGA VIROSA* (ROXB EX. WILLD) IN WISTAR RATS' by Salihu Tanimu TANKO meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

| | | |
|---|---|---|
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## ABSTRACT

*Securinega virosa* is one of the African medicinal plants described as a true "cure all" among traditional practitioners. Different parts of *Securinega virosa* have been used in the management of different disorders in Nigeria and other parts of the world. There is therefore the need to establish the toxicity profile of *Securinega virosa* leaves. In this study, toxicological assessment of the methanol leaf extract of *Securinega virosa* was carried out in Wistar rats. Forty rats of either sex (20 males and 20 females) were divided into four groups of 10 rats (5 males and 5 females) each and kept in separate cages. The rats were administered with 250, 500 and 1000 mg/kg body weight of the extract daily for 28 days. The effect of the extract on body weight, haematolological and biochemical (renal function, liver function and lipid profile) parameters as well as histopathological studies of the organs (liver, kidney, spleen, lungs, brain, heart, stomach, uterus, ovaries and testes) was assessed. The oral and the intraperitoneal median lethal dose ($LD_{50}$) values of the extract were estimated to be greater than 5000 mg/kg bodyweight and within the doses of the study, no mortality was observed in both acute and sub-acute toxicity studies. The extract had no significant ($p$□0.05) effect on body weight. The haematological parameters measured were normal except a significant ($p$□0.05) increase in platelets count observed at the dose of 1000 mg/kg body weight. There was a significant ($p$□0.05) increase in liver biomarkers, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in both male and female rats treated at doses of 500 and 1000 mg/kg body weight when compared with the control. There was also a significant ($p$□0.05) increase in the serum urea and creatinine in these groups. Histopathological findings indicated that there were some levels of liver damage

characterised by centrilobular necrosis peri-portal hepatocytic vacuolation and scattered inflammatory changes, kidney (glomerular damage and atrophy, tubular epithelial cell necrosis and vascular congestion), and brain (degeneration of nerve fibres, pyknosis and vacuolation) at doses of 500 and 1000 mg/kg body weight. Some levels of damage was also observed in the spleen (splenic congestion) and lungs (diffused areas of congestion and inflammation) of both male and female rats across all the doses administered. These findings suggest that the methanol leaf extract of *Securinega virosa* is practically non toxic following acute administration, however, it produced some levels of damage to the liver, kidney, heart, spleen, lungs and brain in Wistar rats following oral administration for 28 days.

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## ABBREVIATIONS

| | |
|---|---|
| **ABU** | Ahmadu Bello University |
| **ALT** | Alanine aminotransferase |
| **ALP** | Alkaline phosphatase |
| **AST** | Aspartate aminotransferase |
| **ANOVA** | Analysis of variance |
| **$ED_{50}$** | Median effective dose |
| **EDTA** | Ethlene diamine tetra-acetic acid |
| **H** | Haematoxylin |
| **E** | Eosin |
| **HDL** | High density lipoprotein |
| **$LC_{50}$** | Median lethal concentration |
| **$LD_{50}$** | Median lethal dose |
| **LDL** | Low density lipoprotein |
| **MCH** | Mean corpuscular haemoglobin |
| **MCHC** | Mean corpuscular haemoglobin concentration |
| **MCV** | Mean corpuscular volume |

| | |
|---|---|
| **MLESV** | Methanol leaf extract of *Securinega virosa* |
| **OECD** | Organisation for economic cooperation and development |
| **OGTT** | Oral glucose tolerance test |
| **OWI** | Organ weight index |
| **PCV** | Packed cell volume |
| **RBC** | Red blood cell |
| **$R_f$** | Retention factor |
| **ROW** | Relative organ weight |
| **Rpm** | Revolutions per minute |
| **SEM** | Standard error of the Mean |
| **CNS** | Central nervous system |
| **TLC** | Thin layer chromatography |
| **USA** | United States of America |
| **WBC** | White blood cell |
| **WHO** | World Health Organisation |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

In the absence of an efficient primary health care system, traditional medicine occupies a central place in the provision of health care, especially among rural communities of developing countries. The strong historical bond between plants and human health is well substantiated by plants species' diversity and related knowledge of their use as herbal medicines (Tibuti and Dhillion,2003). In addition, it is attributable to the accessibility and affordability of herbal medicines (Steenkamp, 2003). The difficult economic conditions often limit access to the rather expensive conventional medicines that are available, with the result that people seek out less expensive alternative medicines such as herbal products (WHO, 2010).

A medicinal plant is any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of therapeutic agents (Sofowora,2008). The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Jordan *et al*., 2010).

A survey conducted by the World Health Organization (WHO) indicated that about 70-80% of the world populations rely on non-conventional medicine mainly of herbal sources in their primary healthcare (WHO, 2003). This is especially the case in developing countries where the cost of consulting a qualified medical doctor and the price of medication are beyond the means of most people (Dyson, 1998; Chan, 2003).

Different retrospective studies done over the last two decades indicated that the incidence of deaths occurring due to exposure to plants (as a proportion of total patients poisoned by traditional plant medicine) was about 1.5% in France, 5% in Belgium, 6.5% in Italy,7.2% in Switzerland and 6% in Turkey (Gaillard and Pepin, 1999). The total number of deaths due to exposure to plants throughout the world however, is very difficult to establish and must certainly be underestimated since all cases of such deaths were, from analytical and forensic points of view, not always well documented and thus, rarely published (Bent and Ko, 2007)

Despite the growing market demand for herbal medicines, there are still concerns associated with not only their use, but their safety.Many plants produce toxic secondary metabolites as natural defence from adverse conditions. In some toxicologically and medicinally relevant plant species like *Digitalis purpurea*, *Hyoscyamus niger*, *Atropa belladonna*, *Physostigma venenosum*, *Podophyllum peltatum*and *Solanum nigrum*, these toxic substances are not distinguished from therapeutically active ingredients (Mosihuzzaman, 2012).

## 1.2 Statement of Research Problem

Herbs and herb-derived medicines have played a crucial role in health and disease management for many centuries. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home (WHO, 2012). The global demand for herbal medicinal products has increased significantly in recent years. It is estimated that, the world's population will be more than 7.5 billion in the next 10 to 15 years. This increase in population will occur mostly in the southern hemisphere, where approximately 80% of the population still relies on a traditional system of medicine based on herbal drugs for primary healthcare (WHO, 2012). Less than 10% of herbal products in the world

market are truly standardized to known active components and strict quality control measures are not always diligently adhered to (Winston, 2007). This raises concern on their safety and implications for their use as medicines. Toxicity testing can reveal some of the risks that may be associated with the use of herbs, therefore avoiding potential harmful effects when used as medicine. Some plant-based drugs have been used for centuries and for some like cardiac glycosides, there is no alternative conventional medicine (Obidike and Salawu, 2013). Therefore, medicinal plants and their bioactive molecules are always in demand and are a central point of research. The earliest report of the toxicity of herbs originated from Galen, a Greek pharmacist and physician who showed that herbs do not contain only medicinally beneficial constituents, but may also be constituted with harmful substances (Cheng and Zhen, 2004).

The most important aspects during the usage of drugs from all sources are their quality, efficacy and safety. Assumptions have been made that the use of herbal drugs in humans is going on without any noticeable toxic effects (Sushma *et al*., 2012). The assumption is not based on scientific evidence but on the fact that herbs are often believed to be safe because they are 'natural' (Glesler, 1992). A study carried out on 548 compounds marketed between 1975 to1999 showed that 10.2% were withdrawn or acquired a black box warning due to toxicity (Boone *et al.*, 2005). This emphasizes the need to carry out toxicity testing on all drugs intended for human and animal consumption.

### 1.3 Justification of the Study

The primary aim of toxicological assessment of any herbal medicine is to identify adverse effects and to determine limits of exposure level at which such effects occur. An equally important objective of toxicity testing is the detection of toxic plant extracts or compounds derived thereof

in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources. This will facilitate the identification of toxicants which can be discarded or modified during the process and create an opportunity for extensive evaluation of saferpromising alternatives (Gamaniel, 2000).

One of the basic criteria set by WHO for the use of herbs as medicines is that they should be shown to be non-toxic (WHO, 2011). Although the use of ethno-medicine is widespread in Africa, most of the plants have not been thoroughly investigated for their toxicities (Sowemimo *et al*., 2007).Besides studies on quality and efficacy of herbal medicines, it is necessary to ensure the safety of a product and this entails toxicity testing. Toxicity testing of herbal drugs has also been found to have a lot of benefits (OECD, 2008). Notably, it is easy to identify the toxic effects and thus determine the limit of exposure levels especially to sensitive population. Once these toxicants are known they may be discarded or modified *via* dosage adjustment, chemical group or structural adjustments (Obidike and Salawu, 2013).

The leaves, stembark and rootbark extracts of *Securinega virosa* have been used in the management of different disorders in Nigeria (Magaji *et al*., 2014; Tanko *et al*., 2008). There is paucity of scientific information on the potential toxicity profile of the leaf extract of *Securinega virosa.* Toxicity data, are therefore required to predict the safety and effect of short and long term exposure to the plant because of its widespread use in the management of various disorders.

### 1.4 Aim of the Study

The aim of this study is to establish the toxicity profile of the methanol leaf extract of *Securinega virosa* in Wistar rats.

## 1.5 Specific Objectives

The specific objectives of the study were to:

1. determine the effect of methanol leaf extract of *Securinega virosa*on body weight changes in rats.
2. evaluate the effect of methanol leaf extract of *Securinega virosa*on some haematological parameters in rats.
3. evaluate the effect of methanol leaf extract of *Securinega virosa* on some biochemical parameters in rats.
4. assess the histological changes on some vital organs of rats exposed to methanol leaf extract of *Securinega virosa*.

## 1.6 Research Hypothesis

**$H_0$ (Null Hypothesis):** The methanol leaf extract of *Securinega virosa*does not produce toxic effects in Wistar rats.

**$H_a$ (Alternate Hypothesis):** The methanol leaf extract of *Securinega virosa* produce toxic effects in Wistar rats.

The null hypothesis will be rejected if *p*-value is  0.05 and the alternative hypothesis will be accepted.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Medicinal Plants

Plants have been selected and used empirically as drugs for centuries, initially as traditional preparations, then as pure active principles, with this knowledge and accumulated practice passing from generation to generation (Taylor *et al*., 2001). Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Abolaji *et al*., 2007). The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and formed an integral part of primary health care in China, Ethiopia, Argentina and Papau New Guinea (Akinyemi *et al.*, 2005). Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life-style related disorders (Agyare *et al.*, 2009).

In principle, herbal medicines have the potential to elicit the same types of adverse reactions as synthetic drugs; the body has no way of distinguishing between ‘‘natural’’ and man-made compounds. Herbal medicines consist of whole extracts of plant parts (for example roots, leaves) and contain numerous potentially active molecules. It is also important to determine whether herbal treatments that have been shown to be as effective as conventional drugs have a better safety profile. Contrary to the belief of most herbalists, long-standing experience is by no means a reliable yardstick when it comes to judging the risk of adverse reactions (Ernst *et al*., 1998).

The reasons for the popularity of herbal medicine are many and diverse. It appears that traditional medicine is not usually used because of an outright rejection of conventional medicine, but more because users desire to control their own health and because they find complementary medicine to be more congruent with their own values, beliefs and philosophical orientations toward health and life (Astin, 1998). An important reason for the increase in use is that consumers (often motivated by the lay press) consider traditional medicine to be "natural'' and assume it is ''safe''. However, this notion is dangerously misleading; adverse effects have been associated with the use of traditionalmedicine (Abbot *et al*., 1996). Furthermore, traditional medicine may not only be directly harmful (for example adverse effects of a herbal formulation), but like other medical treatments have the potential to be indirectly harmful (for example through being applied incompetently, by delaying appropriate effective treatment, or by causing needless expense) (De Smet, 2004).

## 2.2 Constraints in Herbal Medicine

Constraints often encountered in many countries are those related to regulatory status, assessment of safety and efficacy, quality control, safety monitoring, contamination with toxic or hazardous substances, adulteration of herbal products, use of the wrong plant species by mistake and misuse of herbal medicines by either healthcare providers or consumers (WHO, 2005).

### 2.2.1 Reproducibility of biological activity of herbal extracts

One of the major constraints in using plants in pharmaceutical discovery is the lack of reproducibility of activity for over 40% of plants extracts (Cordell, 2000). The activities detected in screens often do not repeat when plants are re-sampled and re-extracted. This problem is largely due to differences in biochemical constituents of plants harvested at different times and

locations, differences in variety, and variations in the methods used for extraction and biological activity determination. Therefore, a strategy should be adopted to evaluate the qualitative and quantitative variations in the content of bioactive phytochemicals of plant material.

### 2.2.2 Toxicity and adverse effects

The general belief is that herbs are safer than pharmaceuticals because they are natural. But the fact is, healing herbs are neither completely safe nor poisonous. Their use in high quantities and over a prolonged period may be injurious to health. Scientific research has demonstrated that many traditionally used herbal medicines are potentially toxic and some are even carcinogenic and mutagenic(DeSaFerrira and Ferrago, 1999). The toxicity benchmarks for herbal drugs therefore depend on purity, herbs containing toxic substances, bioavailability and reported adverse effects (Ahmad *et al*., 2006).

### 2.2.3 Adulteration and contamination

Adulteration and contamination of herbal medicines appear to be common in countries that are lenient with regard to controlling and regulating the use of extracts and compounds from plants. Adulterations in herbal medicines are particularly disconcerting because they are unpredictable. In many cases, adulterated or contaminated herbal medicines can cause significant medical problems (Rotblatt and Zimet, 2002).

## 2.3 Safety Evaluation of Medicinal Plants

Drugs in general need to be safe at the dose recommended for use. For this reason, the safety of drugs needs to be ascertained before approval for use by regulatory authorities in most countries. When found to be toxic, drugs are usually withdrawn from circulation; for example, troglitazone

(Rezulin), a new antidiabetic drug, was removed from the market after close to 100 of the 1.9 million patients treated with the drug suffered liver failure (Chojkier, 2005). Adequate investigation is therefore necessary to ascertain the safe andtoxic doses of a drug.

Medicinal plants are rich in antioxidants and other helpful bioactive constituents.Therefore, use of their various parts for treatments focuses on these properties to the neglect of possible toxicity. Herbal medicines have therefore been considered to be safe and are being used for the treatment of various problems by the general public as well as traditional medicine practioners worldwide (Oduola *et al.*, 2007).

Although toxicity resulting from the use of herbs have been documented in literature on many occasions, the potential toxicity of herbs has not been acknowledged by the general public or by some professional groups of traditional medicine practitioners (O'Hara *et al.*, 1998).

The extent or mode of toxicity of a substance can be affected by many different factors, such as the pathway of administration, the time of exposure, the number of exposures, the physical form of the toxin (solid, liquid, and gas), the genetic makeup of an individual, an individual's overall health, and many others. Many substances regarded as poisons are toxic only indirectly. An example is methanol, which is chemically converted to formaldehyde and formic acid in the liver. It is the formaldehyde and formic acid that cause the toxic effects of methanol exposure.

On the other hand, idiosyncratic toxicity is a leading cause for failure of drugs in clinical testing and it is the most frequent reason for posting warnings, restricting use, or even withdrawal of the drug from the market (Okafor and Ezeigwe, 2005).

## 2.4 Overview of Toxicity and Definition of Key Terms

### 2.4.1 Toxicity

Toxicity is defined as "the potential of a substance to exert a harmful effect on humans or animals, and a description of the effect and the conditions or concentration under which the effect takes place" (Obidike and Salawu, 2013).

In order to support an application for a clinical trial or for the registration of a new drug,it is necessary to satisfy legislation that requires that certain data should be producedfrom a variety of toxicological investigations that show the safety profile of thecompound to which humans may be exposed. Therefore, in the majority of cases ofevaluation of the toxicity of most substances, rodents and non-human primates are first used in preclinical animal safety studies before further studies are done in humans. Theseanimals are mainly used because of their biological similarity to humans that allows themto be regarded as the suitable metabolic models for humans in a broad range ofinvestigations (Loomis and Hayes, 1996; Pascoe, 1983).

Toxicity can bemeasured byits effects on the target organism, organ, tissue or cells. The toxic effects of a substance onanimal physiology can range from minor changes such as reduced weight gain, smallphysiological alteration or change in the levels of circulating hormones, to severe effects inorgan functional loss leading to death. Intermediate levels of toxicity may cause pain andsuffering (Pak *et al*., 2004). During toxicity studies, five major aspects are put intoconsideration. These include organs affected by the chemical, relevance of effect quantification,concentration of chemical to be tested, *in vitro* markers of toxicity that are relevant to thechemical and how to use the data from *in vitro* test for risk assessment. Toxicological studies inthe pharmaceutical field have been growing exponentially. These developments have

beenprompted by discovery of teratogenic effects of drugs such as thalidomide, exposure ofchemicals to the environment and employees and by conduct and assessment of toxicity studiesas part of good manufacturing practice (Traina, 2006).

In the study of herbal remedies, it has been found that toxicity may result from inadvertentsubstitution of one plant species for another. For example, rapidly progressive renal failure resultingin end-stage renal disease has been reported in women who have taken weight-reducing pillscontaining the Chinese herbs *Stephania tetrandra* and *Magnolia officinalis*. This so called Chinese–herb nephropathy is characterized by a pattern of interstitial fibrosis. The cause of thedisease was later noted to be due to inadvertent inclusion of *Stephania fangchi* containing thenephrotoxic and carcinogenic aristolochic acids instead of *S. tetrandra* that contains weightreducing tetrandrine (Nortier, 2000; Rotblatt and Zimet, 2002).

Another reported case on herbal toxicity involved patients using a dietary supplement containingherbal constituents yohimbine in addition to norephendrine, sodium thiosulphate and 3,5-diiodothyronine. All patients developed hepatotoxicity within 3 months, recoveringspontaneously on withdrawal of the supplement (Flavreau *et al*., 2002). A study carriedout in Zimbabwe confirmed an increase in incidences of poisoning by herbal remedy since 1971(Farnsworth, 1998). These facts support the need to have toxicity profiling of all herbal remedies.

Qualitative toxicity assessment of a chemical substance in laboratory animals or *in vitro* toxicitytesting gives information on its potential to cause toxic effects in humans or animals (Descote,1996). To succeed in this exercise, an appropriate choice of control group must be selected,sufficient number of laboratory animals used and good selection of rigorous

experimentalprotocols. Furthermore, the severity of the effect described on major organs and the relevance ofthe mechanisms involved including the variations in different species assist in extrapolation oftoxicological findings from laboratory animals to man (Descote, 1996). During the study, thetarget organ of toxicity in laboratory animals are identified, the mechanism of induced changesare noted and compared to the properties of the target site in man.

Determination of the toxic potential of new compounds constitutes a major part in drugdevelopment and it involves both *in vivo* and *in vitro* toxicological tests. These tests are verycritical in the assessment of the safety of all pharmaceutical products before they are released forgeneral use. Animal models are used in *in vivo* studies as indicators of potential human toxicity (Magnaand Alan, 2007).

Toxicity testing of herbal extracts is carried out on the same principles as the conventionalmedicine. *In vitro* toxicity testing employs the use of models such as the brine shrimp lethalitytest (BST) whereas in *in vivo* methods, animals such as mice or rats are used. The advantage ofthe brine shrimp in toxicity testing is that the shrimp has a lot of homogeneity in eggs and innewly born nauplii which are highly sensitive to chemicals. The eggs are easily available andhatch with ease within 16-24 hours to the nauplii. The nauplii are utilized within 24-48 hourspost hatching. At the 24th hours post exposure of nauplii to the test chemical, the concentrationcausing 50 % lethality ($LC_{50}$) is determined (Vidotto *et al*., 2013).

The BST method is used to screen for bioactivity, anticancer, cytotoxicity, toxicity, pesticidalactivity or gastroprotective action among other pharmacological effects of plant extracts. Brineshrimp lethality is a rapid method that is reliable, inexpensive and convenient as an in-housegeneral bio-assay tool (Meyer *et al.*, 1982). This method is basically used to predict

toxicity andthe results obtained are compared with result of oral acute toxicity in rodents (Cheng and Zhen, 2004). *In vivo*toxicity testing mainly employs the use of rats although other rodents may be used. Dogs andmonkeys are restricted to advanced stages of testing (Cheng and Zhen, 2004).

Organization for Economic Cooperation and Development (OECD) guidelines are used duringacute and sub-acute oral toxicity testing (Diener *et al*., 1995). It is important to optimize theinformation obtained by using the smallest number of animals to comply with animal welfareregulations. Furthermore, it is important to avoid inducing excessive pain or tissue damage in the animals.Pharmaceuticals with irritant or corrosive characteristics should not be administered in concentrations that produce severe toxicity after administration. During toxicity studies, all theanimals should be checked for morbidity, mortality and specific signs of toxicological relevance.For example, neurofunctional and neurobehavioral, opthalmological observation, body-weightand food/water intake should be assessed. The key haematological parameters investigated are mean corpuscularvolume (MCV), mean corpuscular haemoglobin concentration (MCHC), haemoglobin levels,haematocrit levels, packed cell volume (PCV) total and differential leukocytes, erythrocytes andplatelet counts.

Clinical biochemistry is crucial in investigating major toxic effects on organs especially the kidneyand the liver. Some of the parameters include total protein, albumin, totalcholesterol, alanine aminotransferase (ALT), aspatate aminotransferase (AST), alkaline phosphatase,creatinine, urea and electrolytes, which aid in hepatocellular and renal evaluation. Pathological studies andgross necropsy are done by examining the whole body, orifices, body weight and relativeorgan weight changes among others. In addition, histopathological studies are done on adrenals,lung, liver, kidney, testis, ovaries among others (OECD, 2008). These organs

are consideredto be the most important during toxicity studies in rodents and non-rodents (Michael *et al.*,2007).

Taking the weights of organs is necessary because organ to body weight ratios or organ weightindex (OWI) are commonly calculated and are considered more useful when body weights areaffected (Michael *et al*. 2007). By carrying out toxicity tests, the effects of increase in dose onthe mortality and other effects of the lethal dose that kills are determined. Estimating differentlevels of toxicity by use of $LD_{50}$ for instance can help in estimating the probabilities of anoutcome of toxicity for a given individual in a population. The determination of acute, sub-acute, and chronic toxic effects of the test compounds is therefore crucial (Traina, 2006).

### 2.4.2 Acute toxicity

Acute toxicity has been defined as "the ability of a substance to cause severe biologicalharm or death soon after a single exposure or dose; or any poisonous effect resulting froma single short-term exposure to a toxic substance" (OECD, 2008).

An acute toxicity test is a single test that is conducted in a suitable animal species andmay be done for essentially all chemicals that are of any biologic interest. Its purpose isto determine the symptomatology consequent to administration of the compound and todetermine the order of lethality of the compound. The test consists of administering thecompound to the animals on one occasion (Loomis and Hayes, 1996; Timbrell, 2002;Pascoe, 1983).

Furthermore, acute toxicity tests are those designed to determine the effects, which occurwithin a short period after dosing. They serve to establish the lethal dose range of the testsubstance and provide prompt warning if a highly toxic compound is being dealt with(Poole and Leslie, 1989).

They also provide information on the limiting toxicity arisingfrom the pharmacological effects of the compound on target organs and, often, on themaximum dose to be used in subsequent sub-acuteor chronic toxicity studies(Poole and Leslie, 1989).This test is also used to calculatethe median lethal dose ($LD_{50}$) of a substance, using various standardized methods includingLorke's and acute toxic class methods. Exposure routes may be by oral gavage, inhalation/mucosal, dermal; or by injection into the bloodstream, abdomen, or the muscles. Followingadministration of a test product, animals are observed individually at least once during thefirst 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days in the case of delayed toxicities (OECD, 2008).

As usual, all initial acute toxicity tests are performed on either rats or mice because of the low cost, the availability of the animals, and the fact that abundant reference toxicologic data for many compounds in these species are available (Loomis and Hayes, 1996). In addition, these animals generally metabolize compounds in a similar manner to humans and the compounds (including metabolites) may have similar pharmacodynamics in the animals and humans (Krenzelok and Mrvos, 2011). After exposure to single doses of the test compound (or treatment), the animals are monitored for a minimum of 24hrs for any clearly recognized effect (such as changes in locomotor activity; bizarre reactions; sensitivity to pain, sound and touch; changes in social interaction; aggressive behaviour; convulsions; paralysis, etc.) seen, as an index of toxicity, shortly or/and consistently after the administration of the chemical (Timbrell, 2002; Loomis and Hayes., 1996).

However, the most easily recognized and certainly the most significant of effects is that of death and this outcome is usually used as a primary measure of acute toxicity. If the animals appear to be healthy at the end of 24hrs, they are monitored at daily intervals for at least a further one to

two weeks for the appearance of delayed toxicity (Loomis and Hayes, 1996; Pascoe, 1983; Timbrell, 2002).

The information on acute systemic toxicity generated by the acute toxicity testing is used in hazard identification and risk management in the context of production, management, and utilization of chemicals.

The $LD_{50}$ value, (the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period), is currently the starting point for toxicological classification of chemicals. Laboratory mice and rats are the species typically selected for a classic $LD_{50}$ study. Often both sexes must be used for regulatory purposes. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained (Walum, 1998).

Efforts have also been made to develop *in vitro* systems; e.g., it has been suggested that acute systemic toxicity can be broken down into a number of biokinetic, cellular, and molecular elements, each of which can be identified and quantified in appropriate models. The various elements may then be used in different combinations to model large numbers of toxic trial to predict hazard and classify compounds.

Since a great range of concentrations or doses of various chemicals may be involved in the production of harmful effects, the $LD_{50}$ has been used by some authors to devise categories of toxicity on the basis of the amounts of the chemicals necessary to produce harm. An example of such a categorization, along with the respective lethal doses, is given in the Table below.

**Table 2.1 Classification of toxicity based on $LD_{50}$ dose ranges**

| Category | $LD_{50}$ (mg/Kg) | $LD_{50}$ (mg/Kg) | Classification |
|---|---|---|---|
| Extremely toxic | 1 or less | □ 5 | Super toxic |
| Highly toxic | 1 to 50 | 5 to 50 | Extremely toxic |
| Moderately toxic | 50 to 500 | 50 to 500 | Very toxic |
| Slightly toxic | 500 to 5000 | 500 to 5000 | Moderately toxic |
| Practically non toxic | 5000 to 15000 | 5000 to 15000 | Slightly toxic |
| Relatively harmless | □ 15000 | □ 15000 | Relatively harmless |

(Loomis and Hayes, 1996)

### 2.4.3 Sub-acute toxicity

This is repeat-dose study performed to expose any deleterious changes in organ, haematological and biochemical indices that may arise in the course of repeated administration of a test substance, usually ranges from weeks to a few months (OECD, 2008). The terms 'sub-acute' toxicity and 'sub chronic' toxicity can be differentiated on the basis of exposure, the former having a duration period of one month (28-30 days) and the latter ranging from two to three months (Timbrell, 2009). The test product or compound is usually administered daily throughout the test period and at the end of the study, data generated will include general parameters such as daily food consumption, water intake and body weight measurements.

Other specific endpoints of toxicity assessed will additionally include serum biochemical parameters (lipid profile, protein, urea, creatinine, electrolytes, liver transaminases and phosphatase), enzymatic and non-enzymatic liver oxidative stress indicators (thiobarbituric acid reactive substances, reduced glutathione, catalase) and haematological parameters (white blood cells and differentials, red blood cells, haemoglobin, haematocrit, platelets, lymphocytes). Various organs are examined for gross pathological changes and tissue slices obtained from respective organs are prepared for detailed histological examination (Mukinda and Syce, 2007).

Results of many sub chronic toxicity tests of various plant extracts showed that the major organs usually affected are the liver and kidneys. Hepatotoxic and nephrotoxic effects are mostly to be expected, as the liver acts as the main detoxifying organ for chemical substances, while the kidney is a principal route of excretion for many chemical substances in their active and/or inactive forms (Abdulrahman *et al*., 2007).

Liver injury associated with the use of herbal medicine ranges from mild elevation of liver enzymes to fulminant liver failure often requiring a new transplant; and carcinogenesis (Ahmad *et al*., 2006).

The sub-acute toxicity test in the study was based on OECD (2008), repeated dose28-day oral toxicity study in rodents. In this form of toxicity, adverse effects occur as a result ofrepeated daily dosing of a chemical or exposure to the chemical, for part of an organism's lifespanusually not exceeding 10 % of the animals' lifespan. Exposure for 28 days provides a first-hand indicator of potential sub acute toxicity. The test is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on

nervous, immune and endocrine systems. The duration of exposure is normally 28 days in rodents where results are used for hazard identification and risk assessment (OECD,2008). Information gathered from the studies is used in selecting doses for repeat-dose studies as a source of preliminary identification of target organs of toxicity, and may also reveal delayed toxicity. Sub-acute toxicity studies in animals are essential for any pharmaceutical products especially those intended for human use.

### 2.4.4 Chronic toxicity

Chronic toxicity is defined as "the capacity of a substance to cause poisonous health effects in humans, animals, fish and other organisms after multiple exposures occurring over an extended period of time (3-18 months) or over a significant fraction of an animal's or human's lifetime (OECD, 2008).

The purpose of the chronic toxicity test is to investigate the harmful effects foreign compounds that are introduced to animals in repeated doses or in continuous exposure over an extended period of time may produce (Poole and Leslie, 1989). The dose levels of compounds used usually range from a very low fraction of the therapeutically effective dose (i.e. somewhere in the range of the $ED_{50}$ for the compound in that species, or of the same order as the anticipated human therapeutic dose range) to doses that approach the maximum non-lethal dose (as established in rodent acute toxicity studies) (Poole and Leslie, 1989; Loomis and Hayes, 1996).

A central concept of toxicology is that effects are dose dependent; even water can lead to water intoxication when taken in large enough doses, whereas for even a very toxic substance such as snake venom, there is a dose below which there is no detectable toxic effect.

Drugs that exert their toxic effects on the liver may do so through the hepatic production of a toxic metabolite. Drug toxicity may occur in all individuals exposed to a sufficient concentration of a particular drug. A drug may even be toxic in some individuals at concentrations normally tolerated by most patients prescribed the drug. This phenomenon is known as idiosyncratic drug toxicity, and may be due to a genetic or immunologic cause (Maurer, 2006).

## 2.5 Toxicity Aspects of Use of Herbal Products

There is an ongoing world-wide "green" revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Williamson *et al*., 1996). Many writers claim that it is assumed that "all things natural are good" (Gaillard and Pepin, 1999) and, generally, the extensive traditional use of herbal products is not assumed to be based on a comprehensive well documented logic, but rather on empirical wisdom accumulated over many years, often arrived at through trial and error and transmitted orally from generation to generation.

This traditional methodology has enabled those herbal medicines producing acute and obvious signs of toxicity to be recognized and their use avoided. However, the premise that "traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true" (Ojewole, 2004)). The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity, andhepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies(Williamson *et al*., 1996; Tomlinson and Akerele, 1998).

## 2.6 Factors Influencing Toxicity

Human fatalities caused by poisonous plants - especially resulting from accidental ingestion - are rare in the United States of America (Krenzelok and Mrvos, 2011). Unfortunately, there is little or no documentation available in Ghana and most parts of Africa on incidents of fatalities caused by poisonous plants. This is because, almost every plant material ingested is considered medicine or food (Kumar *et al*., 2009).

Generally, the toxicity of plants is caused by the presence of certain compounds such as cyanogenic glycosides and lectins like phytohaemaglutinin (Maurer, 2006).

Many food plants possess toxic parts, are toxic unless processed, or are toxic at certain stages of their life. Notable examples include: Apple (*Malus domestica*) whose seeds contain cyanogenic glycosides; in most species, the amount found in a single fruit won't kill a person; but it is possible to ingest enough seeds to provide a fatal dose (Okonkwo *et al*., 2010).

Other plants with toxic effects include: *Cerbera odollam* (colloquially known as the suicide tree). The nut contains cerberin, which is known to stop the heart (Gaillard *et al.*, 2004). *Helleborus niger* (also known as Christmas rose) contains protoanemonin (Olson, 2000), or ranunculin (Spoerke and Smolinske, 1990), which has an acrid taste and can cause burning of the eyes, mouth and throat, oral ulceration, gastroenteritis and haematemesis (Olson, 2000).

## 2.7 Target Organ Toxicity

The extent to which an organ is susceptible to toxicity varies from organ to organ. For example the kidneys and liver are more highly vascularised making them more susceptible to toxicity than the bone tissues.

### 2.7.1 Blood toxicity

Blood which forms the main medium of transport in the body is a very important tissue that serves to transport many drugs and xenobiotic. Since all foreign compounds are distributed via the bloodstream, the various components, cellular and non-cellular, are initially exposed to significant concentrations of toxic compounds (Timbrell, 2009).

Some plant materials when ingested either in the raw state or their extract, have been reported to cause anaemia which may result from sequestration of red blood cell in the spleen, impaired red cell production or primary bone marrow dysfunction (Cheeke, 1998).

Damage to and destruction of the blood cells results in a variety of consequences such as a reduction in the oxygen-carrying capacity of the blood if the cells affected are the red blood cells. The assessment of blood is relevant to the evaluation of risks since the haematological system carries a higher predictive value for toxicity in humans (90%) (Olson *et al.*, 2000).

### 2.7.2 Kidney toxicity

The kidneys have several functions and serve important regulatory roles in most animals. They are essential in the urinary system in serving homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining salt and water balance) and serving the body as a natural filter of the blood, removing wastes which are diverted to the urinary bladder. The kidneys excrete wastes such as urea and ammonium in urine; they are also responsible for the re-absorption of water, glucose, and amino acids and produce hormones including calcitriol, renin, and erythropoietin.

Exposure to chemical substances can cause adverse effects on the kidney, ureter, or bladder (Kidney Toxicity). The kidney is usually susceptible because of its role in filtering harmful substances from the blood. Toxic injury to the kidney is known to occur as a result of exposures to halogenated hydrocarbons, such as carbon tetrachloride and trichloroethylene, and the heavy metals cadmium and lead. Some of these toxicants cause acute injury to the kidney, while others produce chronic changes that can lead to end-stage renal failure or cancer (Toback, 1992).

### 2.7.3 Liver toxicity

The liver is the main organ of metabolism and energy production. Its other main functions include: bile production, storage of iron, vitamins and trace elements, detoxification, conversion of waste products for excretion by the kidneys. The liver metabolises both beneficial and harmful substances. It has an immense task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Thus, the liver is subjected to a variety of diseases and disorders (which may be acute or chronic) caused by these agents or their metabolites. Acute and chronic liver diseases constitute a global concern, but medical treatments for these diseases are often difficult to handle and have limited efficacy (Kim *et al.*, 2009). It is a unique organ in the sense that a significant loss of liver cells due to drug toxicity or other insults can be overcome by regeneration (Mehendale, 2005).

## 2.8 Phytochemistry

Phytochemicals are chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not established as essential nutrients.

Phytochemical screening of a plant involves the extraction of the plant material, separation and isolation of the constituents and characterization of the isolated compounds. The phytochemical screening of plant materials may reveal the presence of bioactive compounds such as: reducing sugars, alkaloids, flavonoids, tannins, saponins, anthraquinones, terpenes, cardiac glycosides, phenols, steroids, cardenolides and so forth (Rajendra and Estari, 2013).

### 2.8.1 Alkaloids

Alkaloids are a group of complex nitrogen-containing compounds derived from plantsvia complex biosynthetic pathways (Kittakoop *et al*., 2014). Alkaloids are a structurally diverse class of nitrogen containing compounds with over 12,000 structures elucidated from plants (Verpoorte and Memelink, 2002; Wink, 2003). Well known alkaloid compounds include: Purine alkaloids (Caffeine and Theobromine), Tropane alkaloids (Cocaine and Scopolamine), Benzylisoquinoline alkaloids (Berberine and Morphine), Monoterpenoid indole alkaloids derived from Tryptophan (Murasco and Schmidt-Dannert., 2003). Alkaloids have been used as anticancer agents (Vinca alkaloids), antimalarials (Quinine), analgesics (Morphine), central nervoussystem stimulants (Caffeine), muscle relaxants (Hyoscyamine) and so forth (Rathbone and Bruce, 2002).

### 2.8.2 Flavonoids

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. They are present in different plant parts including the leaves, stem, roots, flowers and seeds (Harnafi and Amrani, 2007). They are classified into five major subgroups: flavones, flavonoids, flavanones, flavanols and anthocyanidins (Nijveldt *et al*., 2001). Flavonoids is involved in scavenging free radicals (Nijveldt *et al*., 2001). It has been identified as a potent hypolipidemic agent in a number of studies (Harnafi and Amrani., 2007). The anti-inflammatory, antithrombotic, vasoprotective,

anti-tumour and gastric mucosa protective effects of some groups of medicinal plants have been attributed to the presence of flavonoids (Trease and Evans, 2001).

### 2.8.3 Saponins

Saponins are naturally occurring surface glycosides mainly produced by plants. Saponin derived its name from its ability to form stable soap-like foams in aqueous solutions (Francis *et al*., 2007). Two types of saponins have been identified namely triterpenoids and steroid saponins (Trease and Evans, 2001). Saponinsexhibit antimicrobial activity and have a lytic effect on erythrocyte membrane (Francis *et al*., 2007). A study by Yoshikawu and co-workers demonstrated the hypoglycaemic effect of saponnins (Yoshikawu *et al*., 2001). Saponins have been shown to lower serum cholesterol level in animals (Al-Habori and Raiman, 1998; Matsuura, 2001). Saponins have been reported to be highly toxic to fish because of their damaging effect on the respiratory epithelia (Roy *et al*., 1990). They are considered to be active compounds of many traditionally used fish poisons, like mahua as cake (Francis *et al*., 2007).

### 2.8.4 Tannins

Tannins are polyphenolic compounds of plant origin and are of two types; hydrolysable tannins (polyesters of gallic acids) and condensed tannins (Mc Sweeney *et al*., 2001). They act as antidiarrhoeal agents and have been employed as antidotes in poisoning by heavy metals, alkaloids and glycosides (Trease and Evans, 2001). The antinutritive effects of tannins are associated in their ability to combine with dietary proteins, polymers and minerals thus retarding their digestion. Tannins can also impair digestive processes by complexing with enzymes and endogenous proteins (Mc Sweeney *et al*., 2001).

### 2.8.5 Cardiac glycosides

The aglycone part of cardiac glycoside is a tetracyclic steroid with an attached unsaturated lactone ring that may have 5 or 6 members. Cardiac glycosides are classified into two groups according to the lactone ring: the C23 cardenolides and the C24 bufadienolides. Cardiac glycosides have been used as drugs for the treatment of heart failure e.g digitoxin from digitalis (Padua *et al*., 1999). The heart arresting properties of these glycosides also render them effective as arrow poisons (Trease and Evans, 2001).

### 2.8.6 Anthraquinones

They are present in purgative drugs e.g Senna leaf (Trease and Evans, 2001). A number of new anthraquinones such as kwanzoquinones was isolated from the root bark ofdaylilies.It exhibits*in-vitro* anti-tumour effects against four human cancer cell lines and its cytotoxicity has also been documented (Cichewicz*et al*., 2004).

## 2.9 Chromatography

The word chromatography means "colour writing" which is a way that a chemist can test liquid mixtures. While studying the colouring materials in plants, a Russian botanist invented chromatography in 1903 (Harry and Christopher, 1989).

Chromatography is based on differential migration. It consists of three steps, spotting, development and visualization. In chromatography, there is a mobile phase and a stationary phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to

be tested. As the mobile phase continues to travel through the stationary phase, it takes the compounds with it.

Thin layer chromatography (TLC) is a method for analyzing mixtures by separating thecompounds in the mixture. TLC can be used to help determine the number of components in a mixture as well as identityand purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of areaction (Bhawani *et al*., 2010).

Thin layer chromatography is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents (Bhawani *et al*., 2010).

The retention factor ($R_f$), is a quantitative indication of how far a particular compound travels in a particular solvent. The $R_f$ value is a good indicator of whether an unknown compound and a known compound are similar, if not identical (Bhawani *et al*., 2010). If the $R_f$ value for the unknown compound is close or the same as the $R_f$ value for the known compound then the two compounds are most likely similar or identical.

The retention factor, $R_f$, is defined as:

$R_f$ = distance the solute (D1) moves divided by the distance travelled by the solvent front (D2)

$R_f$ values are between 0 and 0.999 and without units (Bhawani*et al*., 2010).

## 2.10 The Plant: *Securinega virosa*

**Botanical name:** *Securinega virosa*

**Family:** Euphorbiaceae

**Order:** Geraniales

**Genus:** *Securinega*

**Species:** *Securinega virosa* (Roxb.ex Willd.)

**Synonyms:** *Flueggea virosa*

### 2.10.1 Description

*Securinega virosa* (Euphorbiaceae) is a dense, low and many branched shrub or sometimes a small spreading tree up to 6m high, although more commonly 2-3m, ever green or deciduous with a stem up to 8cm in diameter. The bark is grey-brown and smooth. The leaves are extremely variable in shape, elliptic-ovate, obovate or orbicular, 2.5 to 5cm in length, rather glaucous beneath and rounded, obtuse or pointed at the tip. The flowers are usually borne on axillary fascicles. The fruit is mostly small, black or greenish yellow, dry, fragrant and about 3 to 5mm in diameter. It is widely distributed throughout tropical Africa, also in India, Malaya, China and Australia (Dalziel, 1936).

In Nigeria, it is found in virtually all parts of the country where it is commonly referred to:

*"tsuwaawun karee, gussu, gwiiwar karee*" – Hausa

"*iranje"* – Yoruba

"*njisinta*" – Ibo

"*shim shim"* – Kanuri

"*camal, cambe, came*" – Fulani

(Neuwinger, 1996).



**Plate I:*Securinega virosa*in its natural habitat in Samaru, Zaria**

### 2.10.2 Ethnomedicinal uses of *Securinega virosa*

*Securinega virosa* has enjoyed patronage among traditional practitioners and has been described as a "cure it all" in African traditional medicine (Neuwinger, 1996). The plant is one of the great African medicines used for many diseases. In north east Tanzania, the leaves and root decoction are used for malaria, gonorrhoea, sexual impotence, female infertility, epilepsy, diarrhoea and headaches (Neuwinger, 1996). In Senegal, it is used for liver and kidney diseases, genitor-urinary symptoms, venereal diseases and analgesia (Watt and Breyer-Brandwijk, 1962). In Zimbabwe, its roots decoction is used as an aphrodisiac (Dalzel, 1936). In tropical Africa and Philippines, the leaves and roots decoction are used as laxatives, to calm children and help the sleep and to destroy worms in sores (Neuwinger, 1996). The root and fruits are used as snake-bite remedy in Tangayika, present day Tanzania (Watt and Breyer-Brandwijk, 1962). The root decoction is drunk in Ghana for fever and as sedative in children (Moshi *et al*., 2000). The leaves have laxative properties and are taken as decoction (Dalziel, 1936). The leaves decoction is commonly used by traditional medicine healers in Ogun State, Nigeria, for the management of cancer (Soladoye *et al*., 2010). In norh Eastern Nigeria, the root and leafy twigs decoction is used for the treatment of epilepsy and mental illnesses (Magaji *et al*., 2008, Yerima *et al*., 2009).

### 2.10.3 Chemical constituents

Two cytotoxic compounds; Virosecurinine and Viroallosecurinine were isolated from the leaves of *Securinega virosa* (Tatematsu*et al*, 2006). Ahmad and workers (2006) isolated Isocoumarin bergenin in the leaf sample. In addition to bergenin, Kumar and colleagues (2009) extracted gallic acid, ellagic acid, quercetin, β – sitosterol and the known alkaloid Nb-methyltetrahydro-β-carboline from an Indian leaf sample.One new flavonoid glycoside, 3-O-kaempferol 4-O-

(galloyl)-beta-D-glucoside, one new bergenin derivative, 11-0-caffeoylbergenin, were isolated from the leaves of *Securinega virosa* (Sanogo *et al.,* 2009).

The root bark was found to contain an alkaloid "flugeine" (Monkodkaew*et al*., 2009),which was later also isolated from the root bark from Nigeria and identified as hordenine (Iketuboisin and Mathieson, 1963), dihydronorsecurinine, viroallosecurinine (Saito et al., 1964). A novel alkaloid; 14,15-epoxynorsecurinine was isolated from the South African variety of *Securinega virosa* (Dehmlow*et al*., 1999). Bergenin has also been isolated from the root bark of *Securinega virosa* from Nigeria (Magaji *et al*., 2014).

### 2.10.4 Pharmacological effects of theextracts of *Securinega virosa*

Anti-diabetic properties: Studies have shown that the methanol leaf extract of *Securinega virosa*produceda significant reduction in blood glucose levelsin streptozocin-induced diabetes rats (Tanko *et al*., 2008).

Anti-diarrhoeal properties: A studyby Magaji and co-workers investigating the antidiarrheal activity of methanol extracts of the leaves, stem bark and root bark of *Securinega virosa*on castor oil-induced diarrhoeal model showed the leaf and root bark extract to possess pharmacological activity against diarrhoea (Magaji *et al*., 2007).

Anti-microbial activity: In Tanzania, the alcoholic root extract of the plant was tested for anti-microbial activity and found to exhibit activity against*Escherichiacoli* and to a lesser extent against *Staphylococcusaureus*and antifungal activities (Khan *et al*., 1980).

Anti-malarial activity: The methanol leaf extract of *Securinega virosa* exhibited significant antimalarial activity against *Plasmodium falciparum, in vitro*, comparable to quinine used as standard drug (Gbeassor *et al*., 1989).

Analgesic and anti-inflammatory: A study by Yerima and colleagues revealed that the methanol leaf extract of*Securinega virosa* possessed significant analgesic effect and moderate anti-inflammatory activity (Yerima *et al*., 2009).

Behavioural effects/ Sedative properties: Study of methanol root bark extract showed significant and dose-dependent reduction in the onset and prolonged duration of sleep. It also produced significant and dose-dependent motor coordination deficit in mice (Magaji *et al*., 2008). Fractions from a methanolic bark extract of *Securinega virosa* was also found to possess sedative activity (Magaji *et al*., 2007a). Berginin isolated from the root bark of *Securinega virosa* possesses sleep-inducing property (Magaji *et al*., 2015).

Antipsychotic properties:Crude and aqueous fraction of *Securinegavirosa* root bark extract were found to possess antipsychotic properties (Magaji *et al*., 2014) andcentral depressant activity of butanol fraction root bark was also reported (Aiyelero*et al.,* 2012).

Cytotoxic properties: Virosecurinine and viroallosecurinine were isolated as two cytotoxic alkaloidsfrom the leaves of *Securinega virosa*. A comparison of the cytotoxicity of Virosecurinine and several of its derivatives indicates that an α,β- and a γ,δ-unsaturated lactone located in a strained ring system, such as rings -B, -C, and -D of Virosecurinine is structurally required for significant cytotoxicity (Tatematsu *et al*., 2006). Other alkaloids isolated from the root bark of Securinega virosa include hordenine and norsecurinine (Iketubosun and Mathieson, 1963).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Materials

#### 3.1.1 Plant material

Fresh leaves of *Securinega virosa* was collected from a residential area in Samaru, Sabon Gari Local Government Area of Kaduna State, in May 2013. The leaves wereidentified and authenticated by Messr Umar Galla of the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria,Nigeria, by comparing with an existingvoucher specimen (No.918)already deposited at the herbarium.

#### 3.1.2 Animals

Adult Wistar rats of both sexes weighing 120-160g were obtained from the Animal House facility of theDepartment of Pharmacology and Therapeutics, A.B.U, Zaria. The animals were maintained in a well ventilated room and fed with standard laboratory animal feed and clean water provided*adlibitum*. The animals were housed in small groups of five animals of same sex per cage and were allowed to acclimatize to the laboratory environment for a period of one week before the commencement of the experiment. All procedures were done in conformity to standards of laboratory animal care (OECD, 2008).

#### 3.1.3 Chemicals and reagents

Methanol (Sigma-Aldrich, Germany), Hematoxylin and Eosin (B.D.H Ltd., Poole England), Sulphuric acid (May and Baker Limited), Sodium hydroxide (May and Baker Limited),

Fehling’s solution(Merck-Darmstadt, Germany), Mayer’s reagent(Merck-Darmstadt, Germany)

Dragendorff's reagent (Merck-Darmstadt,Germany),Ferric chloride (Merck-Darmstadt, Germany), Ammonium solution(Merck-Darmstadt, Germany), Chloroform (May and Baker), Formalin (May and Baker Limited), Bouin's fluid (May and Baker).

### 3.1.4 Equipment and Glassware

Centrifuge (Multex MSE, England), Weighing balance (Mettler® Model GA110),Olympus microscope (BX-51),Dessicator, EDTA bottles, Glass slides, Plain universal bottles, Cotton wool, Cannula, Pestle and mortar, Animal cages

## 3.2 Methods

### 3.2.1 Preparation of extract

The leaves of *Securinega virosa* were air-dried under the shade at room temperature and subsequently size-reduced into powder with a pestle and mortar. About 1 kg of the powdered leaves was cold maceratedwith 4 litres of 70% methanol exhaustively for 72 hours with occasional shaking. The resultant mixture was filtered through a separating funnel packed with cotton wool at its base. The filtrate was evaporated to dryness on a water bath at 45°Cgivinga greenish residue subsequently referred to as methanol leaf extract of *Securinega virosa* (MLESV). The extract was then stored in a desiccator. Solutions of the extract were freshly prepared for each study.

### 3.2.2 Preliminary phytochemical screening

Phytochemical screening formajor constituents was undertaken using standardqualitative procedures (Trease and Evans, 2001).

*3.2.2.1 Test for reducing sugars (Fehling's Test)*

The aqueous ethanol extract (0.5 g in 5 ml ofwater) was added to boiling Fehling's solution(A and B) in a test tube. The solution wasobserved for a colour reaction.A pink colour indicated the presence of reducing sugars.

*3.2.2.2 Test for anthraquinones (Borntrager's test)*

About 0.5gof the extract was taken in a dry test tube, 10ml of chloroform was added and the mixture shaken for 5 minutes. The mixture was filtered and an equal volume of ammonia solution was added to the filtrate and shaken. A bright pink colour in the upper aqueous layer indicated the presence of free anthraquinones.

*3.2.2.3 Test for terpenoids (Salkowski'test)*

To 0.5 g each of the extract was added 2 ml ofchloroform. Concentrated Hydrogen sulphate (3 ml) wascarefully added to form a layer. A reddishbrown colouration of the interface indicatesthe presence of terpenoids.

*3.2.2.4 Test for flavonoids (Amyl alcohol test)*

Three gram (3g) of the crude extract was macerated in 50 ml of 1% hydrochloric acid and filtered. Four (4) ml of the filtrate was shaken with 5 ml of amyl alcohol. Production of a yellow colour indicated the presence of free flavonoids.

*3.2.2.5 Test for saponins (Frothing test)*

To 0.5 g of extract was added 5 ml of distilledwater in a test tube. The solution was shakenvigorously.and observed for a stablepersistent froth. The frothing was mixed with3 drops of olive oil and shaken vigorouslyafter which it was observed for the formationof an emulsion.

*3.2.2.6 Test for tannins*

Lead Sub-Acetate test: To about 100mg of the extract was added 1 ml of distilled water and mixed after which few drops of lead sub-acetate was added. Formation of Buff-yellow precipitate indicates the presence of tannins.

Ferric chloride test: To about 100mg of the extract was added 2 ml distilled water and shaken, then heated on a water bath at about 400$^{o}$C for 2 minutes. The solution was filtered and to the filtrate was added concentrated tetraoxosulphate (VI) acid and ferric chloride. A blue-black precipitate indicates the presence of tannins.

*3.2.2.7 Test for alkaloids*

Half gram of extract was diluted to 10 ml with acidalcohol, boiled and filtered. To 5 ml of thefiltrate was added 2 ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloidal base. Thechloroform layer was extracted with 10 ml ofacetic acid. This was divided into twoportions. Mayer's reagent was added to one portion and Draggendorff's reagent to theother. The formation of a cream (with Mayer'sreagent) or reddish brown precipitate (withDraggendorff's reagent) was regarded aspositive for the presence of alkaloids.

*3.2.2.8 Test for cardiac glycosides*

Keller-Killiani test: The extract was reduced to dryness, after which 50mg of it was dissolved in 2 ml chloroform. Tetraoxosulphate (VI) acid was added to form a layer, the purple colour formed at the interface indicates the presence of cardiac glycoside.

Kedde's test: To 2 ml of the methanol solution of the extract, 1 ml of 2% 3,5-dinitrobenzoic acid and 1 ml of 5.7% sodium hydroxide was added. An orange colour obtained after five minutes indicates the presence of cardiac glycoside

### 3.2.3 Thin layer chromatography

A Thin Layer Chromatography (TLC) analysis was performed on the methanol leaf extract of *Securinega virosa.* A solution of the extract was applied as a spot near on one end of a silica gel plate. The plate was placed in a tank containing n-butanol-acetic acid-water in the ratio 4:1:5 as eluting solvent systems and allowed for about 2 minutes to move up the plate to effect separationof the components. The plate was then removed and dried. Spots on TLC plates were visualized by spraying sulphuric acid and placed in an oven for about five minutes. The positions of the various bioactive components were marked and their $R_f$values calculated (Harbone, 1973).

### 3.2.4 Acute toxicity study

The median lethal dose ($LD_{50}$) of the extract was determinedorally and intraperitoneally in rats according to Lorke's method (1983).The study was carried out in two phases.

In the first phase, nine rats were randomized into three groups,each consisting of three rats and were given 10, 100 and 1000mg/kg body weight of the extract orally. The rats were kept under the same conditions and observed for signs of toxicity which included but not limited to paw-licking, stretching, respiratory distress and mortality for the first 4 hours and then thereafter for 24 hours.

In the second phase of the study, three rats were placed into 3 group of one rat each. The animals were orally administered 1600, 2900, and 5000mg/kg of the extract respectively based on the result obtained from the first phase. The rats were also observed for signs of toxicity and mortality for the first critical 4 hours and thereafter for 24 hours. The oral median lethal dose was calculated as the square root of the product of the highest non-lethal dose and the lowest lethal

dose, that is, geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

The same procedure was repeated in rats using the intraperitoneal route of drug administration.

### 3.2.5 Sub-acute toxicity study

Sub-acute toxicity study was carried out in accordance with OECD 407 (2008) guidelines. Forty rats of either sex (20 males and 20 females) were divided into four groups of 10 rats (5 males and 5 females) each and kept in separate cages. The females were nulliparous and non pregnant. Group 1, which served as the control receiveddistilled water, while rats in groups 2, 3 and 4 received250, 500 and 1000mg/kgbody weight oral doses of the extract respectively for 28 days. The animals were fasted overnight prior to the first dosing. Following the period of fasting, the animals were weighed before the test substance was administered via the oral route. The animals were observed daily for general symptoms and signs of toxicity. All the rats were allowed free access to food and water throughout the duration of the study.

*3.2.5.1 Effect of the methanol leaf extract of Securinega virosa on body and organweight*

Rats in all the groups were weighed once every week during the period of treatment and on the last day of the study. On the twenty ninth day, the rats were weighed, anesthetized with chloroform and sacrificed.The animals were dissected and the brain, heart, spleen, liver, lungs, kidneys, and testes or uterus were removed, weighed and observed macroscopically. The ovary was exteriorized by grasping the peri-ovarian fat with tissue forceps and excised between the uterine horn and the fallopian tube. The pad of fat was trimmed off and the paired organs weighed together.

The relative organ to body weight ratio (ROW) of each rat was calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight (g) of rats on sacrifice day}}$$

3.2.5.2 *The effects of methanol leafextract of Securinega virosa on haematological parameters*

After euthanasia, 5 ml of whole blood was collected from the jugular vein of each rat by decapitation. The blood samples for the haematological tests were collected into vacuum tubes containing EDTA as anticoagulant and taken to the laboratory. The blood sample for each rat was smeared on a glass slide and stained with Giemsa stain, then analyzedfor the following parameters: packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelets, white blood cell (WBC) and differential count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobinconcentration (MCHC).

The packed cell volume was determined by collecting the blood into a capillary tube, centrifuged and read using the Haematocrit meter.

Leucocytes are distinguished according to their staining reactions and colours produced. Monocytes when stained were pale violet; Neutrophils had light pink colour in the cytoplasms and purple violet in the lobe nucleus; Lymphocytes showed a deep purple stain in the nuclei while their cytoplasm stain a pale blue; Eosinophils stain similar to Neutrophils but are a little paler containing many large round or oval deep orange-pink granules; Basophils have a kidney shaped nuclei and their cytoplasms contain masses of large granules which stain deep purple frequently obscuring the nuclei.

The haemoglobin concentration was determined by placing a drop of blood on the side of the glass wedge of the haemoglobinometer and the red blood cells were haemolysed. The glass

wedge was then fixed into the haemoglobinometer with the eye piece of the haemoglobinometer focused, while the scale was gradually moved to get the haemoglobin value.

The mean cell volume (MCV) was calculated by dividing the value of PCV by RBC count.

The mean cell haemoglobin (MCH) was derived from the value of Hb divided by RBC count.

The mean cell haemoglobin Concentration (MCHC) was derived from Hb and PCV from the formula:$MCHC(g/l) = \frac{\text{Hb (g/l)}}{\text{PCV(l/l)}}$

*3.2.5.3 The effect of methanol leafextract of Securinega virosa on some biochemical parameters*

About 3 ml of blood was further collected from the euthanized and decapitated rats without an anticoagulant. The blood samples for biochemical analyses were collected into plain universal bottles, allowed to clot and centrifuged at 3500rpm for 10 minutes. The sera was separated and stored at -4°C. The biochemical parameters evaluated included: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total and conjugated bilirubin, Total serum protein, serum electrolytes, urea,creatinine, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides.

Alanine aminotranferase (ALT)and aspartate aminotransferase concentrations were determined by putting the serum into two test tubes labelled blank (containing 0.1ml distilled water) and sample (containing 0.1ml of serum). About 0.5ml of buffer solution (100.0mmol/l), L-alanine (100.0mmol/l and oxoglutarate (2.0mmol/l) was added. They were mixed and incubated for 30 minutes at 37°C. Then to each tube was added 0.5ml of 2,4-dinitrophenylhydrazine solution (2mmol/l) which was mixed and allowed to stand for 20 minutes at 20 - 25°C. Finally, 5ml sodium hydroxide was added into each tube, mixed and the absorbance of sample ($A_{sample}$)

measured against the reagent blank after 5 minutes at wavelength 546nm by colorimetric method(Reitman and Frankel,1957).

Alkaline phosphatase (ALP): King-Armstrong method: Serum (0.05ml) was pipetted into a cuvette and 3.0ml of reagent (10mmol/l p-nitrophenylphosphate) was added. This was mixed and the initial absorbance read at 405nm and the timer was started simultaneously. Readings were taken again after 1,2 and 3 minutes.

Total bilirubin: Two (2) cuvettes were labelled as blank and sample (0.2mls of serum), and to each was added 0.2ml of sulphanilic acid (29mmol/l), hydrochloric acid (0.17mmol/l),caffeine (0.26mmol/l) and sodium benzoate (0.52mmol/l). To the sample cuvette was added 0.05ml of sodium nitrite (25mmol/l). This was mixed and allowed to stand for 10 minutes at 20 to 25°C. Then 1.0ml of tartrate (0.93mmol/l), sodium hydroxide (1.9mmol/l) was added with mixing and allowed to stand for 5-10 minutes at 20-25°C and the absorbance of the sample read against the reagen blank ($A_{TB}$) at 560-600nm (King*et al*., 1964)

Urea: Three (3) test tubes were labelled blank, standard and sample; and into each was pipetted 100µl of solution containing ethylene-diamine tetra acetate (EDTA), 116mmol/l; sodium nitroprusside, 6mmol/l and urease, 1g/l. Then 10µl of distilled water was added to the blank test tube, 10µl of the prepared standard to the standard test tube and 10µl of serum to the sample test tube. This was mixed and incubated at 37°C for 10 minutes then to each tube was added 2.5ml of phenol, 120mmol/l, sodium hypochlorite,27mmol/l, sodium hydroxide,0.14mmol/l and standard, 80mg/dl. This was mixed immediately, incubated at 37°C for 15 minutes and the absorbance of the sample ($A_{sample}$) and standard ($A_{standard}$) read against the blank at 546nm (530-570nm). (King*et al*., 1964)

Creatinine: Serum (1.0ml) was deprotenized by adding 1.0ml of trichloroacetic acid and mixing well with a glass rod to evenly disperse the precipitate. This was then centrifuged at 2500 rpm for 10 minutes and the supernatant collected. Then into 3 test tubes labelled blank, standard and sample was pipetted 0.5ml distilled water, 0.5ml standard and 0.5ml supernatant respectively. To each tube was added1.0ml of reagent mix (standard, 2mg/dl; picric acid 35mmol/l and sodium hydroxide 1.6mmol/l). This was mixed, allowed to stand for 20 minutes at 25°C and the absorbance of sample ($A_{sample}$) measured against the blank at 546nm (King-Armstrong, 1964).

#### *3.2.5.4 Histopathological assessment ofsome organs of Rats exposed to methanol leafextracts of Securinega virosa*

Tissue slices of 3 to 4cm thick of the kidneys, liver, stomach, heart, spleen, ovaries, uterus and testes were cut and fixed in labelled containers containing 10% formalin while the brain was fixed in Bouin's fluid. Theywere then dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90% and 100% v/v. The dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections was dehydrated in xylene and rehydrated in the following grades of alcohol: 100%, 90% and 70% v/v and subsequently infiltrated with molten paraffin wax. They were then stained in Haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in Scotch's tap water and stained with Eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100% v/v, and finally cleared in a pox. The slides were then examined microscopically for pathological lesions. Photomicrographs of the lesions were taken at ×100 and ×400 magnifications (Auwioro *et al.*, 2010).

### 3.3 Statistical Analysis

All the quantitative data were expressed as the Mean ± Standard Error of the Mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, apost-hoc Dunnets testwas performed for multiple comparisons and Bonferroni test. A *p*-value less than0.05 was considered significant.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Plant Yield

The percentage yield of the plant extract was 14.3% w/w

### 4.2 Phytochemical Screeningof the Methanol Leaf Extract of *Securinega virosa*

#### 4.2.1 Phytochemical constituents

Phytochemical analyses of the methanol leaf extract of *Securinega virosa* revealed the presence of flavonoids, tannins, steroids, saponins, alkaloids,reducing sugars,cardiac glycosides and anthraquinones (Table 4.1).

**Table 4.1: Phytochemical Constituents of Methanol Leaf Extract of *Securinega virosa***

| TEST | RESULT |
|---|---|
| Flavonoids | Present |
| Tannins | Present |
| Steroids | Present |
| Saponins | Present |
| Alkaloids | Present |
| Cardiac glycosides | Present |
| Anthraquinones | Present |
| Reducing sugars | Present |

### 4.2.2 Thin layer chromatography of methanol leafextract of *Securinega virosa*

The thin layer chromatography revealed the presence of compounds when developed in different solvent system. The thin layer chromatographic plates are shown below (plate II)

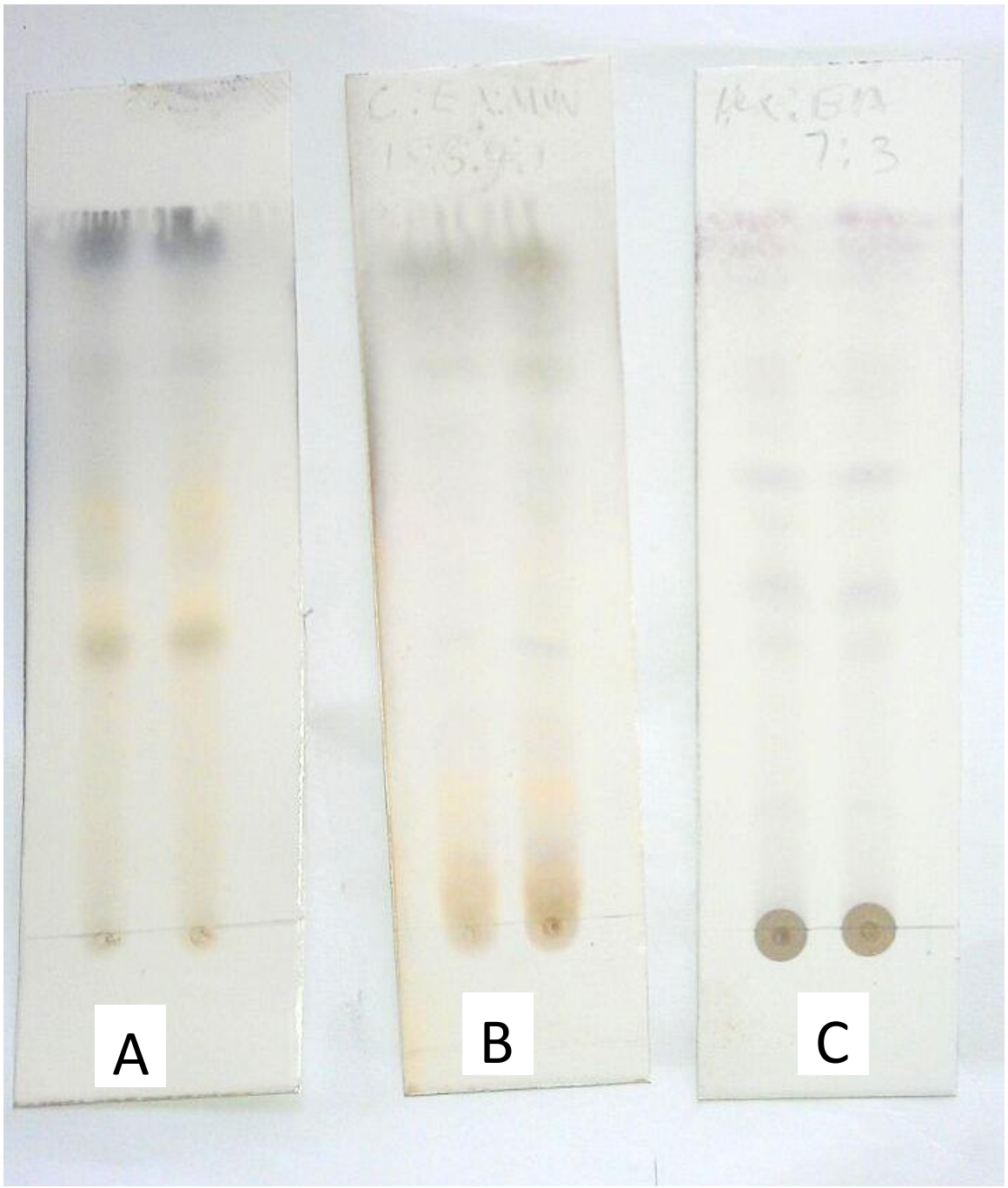



**Plate II:** Thin Layer Chromatographic Plates of methanol leaf extract of *Securinega virosa*

**Table 4.2: Thin Layer Chromatography Profile of Methanol Leaf Extract of *Securinega virosa***

| Solvent System | Colours | $R_f$ Values |
|---|---|---|
| Butanol:Acetic acid:Water(A) | Green | 0.45 |
| | Pink | 0.71 |
| | Grey | 0.82 |
| Chloroform:Ethylacetate:Methanol(B) | Pink | 0.19 |
| | Grey | 0.38 |
| | Violet | 0.79 |
| Hexane:Ethylacetate(C) | Grey | 0.45 |
| | Violet | 0.71 |
| | Violet | 0.82 |

$R_f$= retention factor

## 4.3 Median Lethal Dose ($LD_{50}$) Values

The oral and intraperitoneal median lethal dose ($LD_{50}$) of the methanol leaf extract of *Securinegavirosa* in rats was found to be greater than 5000mg/kg body weight. The rats showed no sign of toxicity up to the highest dose administered (Table 4.3).

**Table 4.3 Oral and Intraperitoneal Median Lethal dose ($LD_{50}$) Values of the MethanolLeaf Extract of *Securinega virosa* in Wistar Rats**

| **Routeof Administration** | **Median Lethal Dose ($LD_{50}$) (mg/dl)** |
|---|---|
| **Oral** | □ 5000 |
| **Intraperitoneal** | □ 5000 |

## 4.4 Sub-Acute Toxicity Studies

The oral administration of methanol leaf extract of *Securinega virosa*at 250, 500 and 1000mg/kg produced no observable overt signs and symptoms of toxicity throughout the 28-day study period. All the animals survived throughout the period of the experiment.

### 4.4.1 Effect of methanol leafextract of *Securinega virosa* on body weight (g) of Wistar Rats after Oral Administration for 28 Days

There was a steady increase in weight of both the male and female rats in all the groups over the 28 day period. However, a decrease in weight that was not significant ($p$ 0.05) was observed in the group treated with 1000 mg/kg body weight when compared with the control group in both male and female animals in the $4^{th}$ week of the study period MLESV(Table 4.4)

**Table 4.4: Effect ofMethanol Leaf Extract of *Securinega virosa* on Body Weight (g) in ratsafter Oral Administration for 28 Days**

| Treatment | (mg/kg) | Pretreatment | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|---|---|
| | **Male** | | | | | |
| **D/Water** | **2 (ml/kg)** | 125.75 ± 2.43 | 136.50 ± 3.86 | 150.00 ± 5.58 | 161.25 ± 3.96 | 177.25 ± 2.56 |
| **MLESV** | **250** | 137.00 ± 1.68 | 149.75 ± 1.75 | 160.75 ± 2.49 | 170.25 ± 1.54 | 181.50 ± 1.56 |
| **MLESV** | **500** | 137.00 ± 2.48 | 145.75 ± 2.10 | 154.75 ± 1.65 | 165.00 ± 2.74 | 173.00 ± 2.35 |
| **MLESV** | **1000** | 132.75 ± 1.49 | 139.75 ± 1.11 | 146.25 ± 1.11 | 152.50 ± 1.32 | 163.25 ± 1.25 |
| | **Female** | | | | | |
| **D/WATER** | **2 (ml/kg)** | 111.50 ± 1.32 | 122.75 ± 0.75 | 134.50 ± 1.32 | 146.25 ± 1.37 | 156.75 ± 0.86 |
| **MLESV** | **250** | 125.50 ± 2.10 | 134.25 ± 2.63 | 145.00 ± 2.04 | 154.00 ± 2.04 | 164.00 ± 2.42 |
| **MLESV** | **500** | 121.75 ± 2.06 | 128.50 ± 1.50 | 134.75 ± 1.11 | 140.50 ± 0.64 | 146.50 ± 1.76 |
| **MLESV** | **1000** | 119.25 ± 1.75 | 125.00 ± 2.16 | 130.50 ± 2.32 | 135.25 ± 1.93 | 144.50 ± 2.33 |

Data were analyzed using repeated measures ANOVA followed by Bonferroni test

Values are expressed as Mean ± SEM, n=10

MLESV= Methanol Leaf Extract of *Securinega virosa*, D/water = Distilled water

### 4.4.2 Effect of methanol Leafextract of *Securinega virosa* on relative organ weights (g) in rats after oral administration for 28 days

The relative organ weights of the treated groups were not significantly different from the control except the significant ($p$ 0.05) increase observed in the liver and kidneys of both male and female rats in the groups treated with 1000mg/kg body weight when compared with the control (Tables 4.5 and 4.6).

**Table 4.5: Effects of Methanol Leaf Extract of *Securinega virosa* on Relative Organ Weights(g)in Male Rats afterOral Administration for 28 Days**

| Treatment Group | (mg/kg) | Kidney | Liver | Stomach | Heart | Brain | Spleen | Lungs | Testes |
|---|---|---|---|---|---|---|---|---|---|
| D/Water | 2 (ml/kg) | 1.24 ± 0.08 | 5.89 ± 0.03 | 1.64 ± 0.08 | 0.96 ± 0.02 | 1.87 ± 0.02 | 0.98 ± 0.02 | 2.28 ± 0.06 | 2.18 ± 0.02 |
| MLESV | 250 | 1.26 ± 0.01 | 5.70 ± 0.27 | 1.57 ± 0.04 | 0.89 ± 0.06 | 1.84 ± 0.13 | 0.97 ± 0.06 | 2.18 ± 0.14 | 2.20 ± 0.13 |
| MLESV | 500 | 1.22 ± 0.05 | 5.64 ± 0.14 | 1.60 ± 0.10 | 0.82 ± 0.07 | 1.74 ± 0.13 | 0.89 ± 0.05 | 2.38 ± 0.12 | 2.24 ± 0.19 |
| MLESV | 1000 | 1.78 ± 0.03* | 6.46 ± 0.05* | 1.66 ± 0.02 | 0.92 ± 0.01 | 1.81 ± 0.02 | 0.92 ± 0.01 | 2.30 ± 0.03 | 2.26 ± 0.03 |

Values are expressed as Mean ± SEM; n=10; D/water = Distilled water MLESV= Methanol Leaf Extract of *Securinegavirosa*

*Significantly different from control at $p$ 0.05

**Table 4.6: Effects of Methanol Leaf Extract of *Securinega virosa* on Relative Organ Weights(g) in Female Rats after Oral Administration for 28 Days**

| Treatment Group | (mg/kg) | Kidney | Liver | Stomach | Heart | Brain | Spleen | Lungs | Uterus | Ovaries |
|---|---|---|---|---|---|---|---|---|---|---|
| **D/Water** | **2 (ml/kg)** | 0.87 ± 0.04 | 4.76 ± 0.04 | 1.07 ± 0.03 | 0.95 ± 0.02 | 1.11 ± 0.02 | 0.84 ± 0.02 | 0.91 ± 0.01 | 0.60 ± 0.01 | 0.42 ± 0.01 |
| **MLESV** | **250** | 0.82 ± 0.03 | 4.68 ± 0.10 | 1.09 ± 0.04 | 0.88 ± 0.05 | 1.18 ± 0.06 | 0.86 ± 0.04 | 0.99 ±0.04 | 0.59 ± 0.05 | 0.45 ± 0.03 |
| **MLESV** | **500** | 0.79 ± 0.03 | 4.16 ± 0.09 | 1.01 ± 0.05 | 0.79 ± 0.06 | 1.21 ± 0.08 | 0.81 ± 0.06 | 0.90 ± 0.04 | 0.63 ± 0.02 | 0.43 ± 0.02 |
| **MLESV** | **1000** | 1.83 ± 0.04* | 5.57 ± 0.09* | 1.17 ± 0.04 | 0.93 ± 0.02 | 1.13 ± 0.03 | 0.92 ± 0.02 | 0.93 ± 0.04 | 0.68 ± 0.01 | 0.47 ± 0.01 |

Values are expressed as Mean ± SEM; n=10; D/water = Distilled water MLESV= Methanol Leaf Extract of *Securinegavirosa*

*Significantly different from control at $p$ 0.05

### 4.4.3 Effect of methanol Leafextract of *Securinega virosa* on haematological parameters in Wistar rats after oral administration for 28 days

Sub-acute oral administration of the extractshowed no significant ($p$ 0.05) difference in the red blood cells, haemoglobin, packed cell volume, MCV, MCH and MCHC in all the treatment groups in both male and female ratswhen compared with the control (Table 4.7).

**Table 4.7: Effect of Methanol Leaf Extract of *Securinega virosa* on Haematological Parameters in Wistar Rats after Oral Administration for 28 Days**

| | Treatment | (mg/kg) | RBC(x$10^9$/l) | Hb(g/dl) | PCV(%) | MCV(fl) | MCH(pg) | MCHC(g/dl) |
|---|---|---|---|---|---|---|---|---|
| **Male** | **D/Water** | **2 (ml/kg)** | 8.45 ± 0.06 | 14.25 ± 0.06 | 42.50 ± 0.29 | 5.03 ± 0.02 | 1.68 ± 0.01 | 0.34 ± 0.03 |
| | **MLESV** | **250** | 8.35 ± 0.11 | 13.98 ± 0.20 | 39.75 ± 0.75 | 5.16 ± 0.17 | 1.72 ± 0.05 | 0.34 ± 0.03 |
| | **MLESV** | **500** | 8.33 ± 0.16 | 14.13 ± 0.32 | 42.25 ± 0.85 | 5.38 ± 0.22 | 1.97 ± 0.07 | 0.33 ± 0.03 |
| | **MLESV** | **1000** | 8.38 ± 0.08 | 14.00 ± 0.13 | 42.00 ± 0.41 | 5.49 ± 0.12 | 1.83 ± 0.04 | 0.33 ± 0.03 |
| **Female** | **D/Water** | **2 (ml/kg)** | 7.75 ± 0.16 | 13.55 ± 0.13 | 40.25 ± 0.48 | 5.60 ± 0.12 | 1.75 ± 0.04 | 0.34 ± 0.03 |
| | **MLESV** | **250** | 7.48 ± 0.18 | 13.23 ± 0.14 | 39.38 ± 0.29 | 5.65 ± 0.16 | 1.89 ± 0.05 | 0.34 ± 0.03 |
| | **MLESV** | **500** | 7.55 ± 0.12 | 13.25 ± 0.16 | 39.35 ± 0.87 | 5.57 ± 0.24 | 2.07 ± 0.05 | 0.34 ± 0.05 |
| | **MLESV** | **1000** | 7.84 ± 0.20 | 12.63 ± 0.29 | 37.25 ± 0.75 | 5.05 ± 0.33 | 2.08 ± 0.12 | 0.34 ± 0.03 |

Values are expressed as mean ± SEM; n=10; D/water = Distilled water

MLESV= Methanol Leaf Extract of *Securinegavirosa*

RBC = Red Blood Cells
HB = Haemoglobin
PCV = Packed Cell Volume
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Haemoglobin Concentration
Pg = Picograms
fl = femtolitres

### 4.4.4 Effect of methanol leafextract of *Securinega virosa* on white blood cells and differentialparameters in Wistar rats after oral administration for 28 days

There was a significant increase ($p$ 0.05) in platelets count in both male and female groups treated with 1000mg/kg body weight when compared with control group. The white blood cells and differentials were not significantly different from the control at all the doses tested (Table 4.8).

**Table 4.8:Effect of Methanol Leaf Extract of *Securinega virosa* on White Blood Cells and Differential Parameters in Wistar Rats after Oral Administration for 28 Days**

| | Treatment | (mg/kg) | Platelets ($\times 10^9$/L) | WBC ($\times 10^9$/L) | Neut(%) | Lymph(%) | Mono(%) | Eosin(%) | Baso(%) |
|---|---|---|---|---|---|---|---|---|---|
| **Male** | **D/Water** | **2(ml/kg)** | 154.75 ± 1.89 | 6.30 ± 0.23 | 54.75 ± 1.55 | 49.25 ± 1.25 | 3.50 ± 0.65 | 2.25 ± 0.25 | 1.50 ± 0.29 |
| | **MLESV** | **250** | 151.00 ± 1.68 | 6.18 ± 0.13 | 55.25 ± 1.25 | 47.00 ± 0.91 | 3.25 ± 0.48 | 2.50 ± 0.29 | 2.50 ± 0.29 |
| | **MLESV** | **500** | 153.75 ± 1.75 | 6.08 ± 0.14 | 54.75 ± 1.38 | 48.00 ± 1.29 | 3.75 ± 0.63 | 2.22 ± 0.25 | 1.75 ± 0.48 |
| | **MLESV** | **1000** | 187.00 ± 2.54* | 6.10 ± 0.20 | 52.50 ± 1.56 | 48.75 ± 1.31 | 3.00 ± 0.71 | 2.25 ± 0.48 | 1.50 ± 0.29 |
| **Female** | **D/Water** | **2 (ml/kg)** | 166.50 ± 3.97 | 5.48 ± 0.11 | 52.75 ± 1.11 | 46.00 ± 1.08 | 4.15 ± 0.48 | 2.25 ± 0.48 | 2.23 ± 0.41 |
| | **MLESV** | **250** | 163.50 ± 1.04 | 5.85 ± 0.10 | 47.25 ± 1,65 | 47.00 ± 2.20 | 3.75 ± 0.48 | 2.35 ± 0.25 | 2.25 ± 0.25 |
| | **MLESV** | **500** | 168.25 ± 1.49 | 5.05 ± 0.16 | 49.00 ± 1.29 | 46.75 ± 1.70 | 3.50 ± 0.65 | 2.50 ± 0.29 | 2.22 ± 0.25 |
| | **MLESV** | **1000** | 175.25 ± 2.66* | 5.43 ± 0.09 | 51.50 ± 1.94 | 47.00 ± 1.58 | 4.25 ± 0.63 | 2.75 ± 0.48 | 2.24 ± 0.13 |

Values are expressed as Mean ± SEM; n=10; D/water = Distilled water; MLESV= Methanol Leaf Extract of *Securinegavirosa*

WBC = white blood cell
Neut = Neutrophils
Lymph = Lymphocytes
Mono = Monocytes
Eosin = Eosinophils
Baso = Basophils

### 4.4.5 Effect of methanol leafextract of *Securinega virosa* on renal indices ofWistar rats after oral administration for 28 days

There was no significant change in the serum electrolytes of both male and female rats in the treatment groupwhen compared with the control group. However, therewas asignificant ($p$<0.05) increase in the serum urea and creatinine in both male and female rats in groups treated with 500and 1000mg/kg body weight when compared with control group(Table4.9).

**Table 4.9: Effect of Methanol Leaf Extract of *Securinega virosa* on Renal Indices of Wistar RatsAfter Oral Administration for 28 Days**

| | Treatment | (mg/kg) | Sodium (mmol/l) | Potassium (mmol/l) | Chloride (mmol/l) | Bicarbonate (mmol/l) | Urea (µmol/l) | Creatinine (µmol/l) |
|---|---|---|---|---|---|---|---|---|
| **Male** | **D/Water** | **2 (ml/kg)** | 121.00 ± 0.41 | 4.28 ± 0.08 | 99.00 ± 0.71 | 27.25 ± 0.48 | 6.70 ± 0.11 | 123.00 ± 0.71 |
| | **MLESV** | **250** | 120.50 ± 0.29 | 4.25 ± 0.10 | 98.00 ± 0.91 | 26.25 ± 0.85 | 6.85 ± 0.03 | 125.75 ± 0.63 |
| | **MLESV** | **500** | 119.00 ± 0.91 | 4.26 ± 0.11 | 101.50 ± 1.32 | 27.00 ± 0.91 | 8.10 ± 0.08* | 136.75 ± 0.48* |
| | **MLESV** | **1000** | 122.75 ± 0.85 | 4.31 ± 0.04 | 104.75 ± 0.85 | 27.31 ± 0.85 | 9.50 ± 0.09* | 162.00 ± 0.82* |
| **Female** | **D/Water** | **2 (ml/kg)** | 117.75 ± 0.48 | 4.30 ± 0.08 | 85.50 ± 0.65 | 29.50 ± 0.65 | 6.43 ± 0.08 | 118.00 ± 1.08 |
| | **MLESV** | **250** | 118.75 ± 1.10 | 4.26 ± 0.04 | 85.25 ± 0.85 | 29.75 ± 0.48 | 6.55 ± 0.07 | 120.25 ± 0.85 |
| | **MLESV** | **500** | 117.50 ± 0.65 | 4.31 ± 0.09 | 85.75 ± 0.63 | 30.10 ± 0.71 | 7.28 ± 0.16* | 129.25 ± 0.48* |
| | **MLESV** | **1000** | 120.75 ± 1.18 | 4.33 ± 0.05 | 86.15 ± 0.85 | 30.25 ± 0.41 | 8.75 ± 0.06* | 149.50 ± 1.04* |

Values are expressed as Mean ± SEM; n=10; D/water = Distilled water; MLESV= Methanol Leaf Extract of *Securinegavirosa*

*Significantly different from control at $p \square 0.05$

### 4.4.6 Effect of methanol leafextract of *Securinega virosa* on hepatic indices of Wistar rats after oral administration for 28 days

There was asignificant ($p$□0.05) increase in the liver biomarkers, aspartate amino transferase (AST), alanine amino transferase(ALT) and alkaline phosphatise (ALP) in both male and female rats treated with 500and1000mg/kg body weight when compared withcontrol. However, the serum total protein, albumin, direct and indirect bilirubin were not significantly different for all testeddoses of the extractwhen compared with their respective control (Table 4.10).

**Table 4.10: Effect of Methanol Leaf Extract of *Securinega virosa* on Hepatic Indices of Wistar RatsAfter Oral Administration for 28 Days**

| | Treatment | (mg/kg) | AST (U/l) | ALT (U/l) | TPr (g/l) | Alb (g/l) | ALP (U/l) | DBil (µmol/l) | IndBil (µmol/l) |
|---|---|---|---|---|---|---|---|---|---|
| **MALE** | **D/Water** | **2 (ml/kg)** | 118.50 ± 0.65 | 35.00 ± 0.91 | 79.75 ± 0.25 | 41.10 ± 0.41 | 34.25 ± 0.63 | 15.23 ± 0.14 | 3.48 ± 0.05 |
| | **MLESV** | **250** | 120.50 ± 0.65 | 39.25 ± 0.85 | 78.75 ± 1.31 | 40.20 ± 0.82 | 38.25 ± 0.75 | 15.73 ± 0.06 | 3.78 ± 0.06 |
| | **MLESV** | **500** | 131.05 ± 0.85* | 47.00 ± 1.95* | 79.50 ± 2.75 | 41.75 ± 1.38 | 44.75 ± 0.48 | 15.20 ± 0.13 | 3.08 ± 0.06 |
| | **MLESV** | **1000** | 137.50 ± 1.04* | 57.25 ± 5.23* | 78.00 ± 1.41 | 40.50 ± 0.65 | 63.50 ± 1.4* | 15.88 ± 0.09 | 3.45 ± 0.07 |
| **FEMALE** | **D/Water** | **2 (ml/kg)** | 117.00 ± 0.91 | 34.50 ± 1.32 | 73.25 ± 1.60 | 37.25 ± 0.63 | 23.75 ± 0.48 | 15.00 ± 0.09 | 3.30 ± 0.04 |
| | **MLESV** | **250** | 120.75 ± 0.85 | 35.00 ± 1.68 | 77.25 ± 1.49 | 40.50 ± 1.04 | 29.00 ± 1.08 | 15.53 ± 0.11 | 3.55 ± 0.07 |
| | **MLESV** | **500** | 129.25 ± 2.02* | 39.75 ± 1.11* | 74.50 ± 0.65 | 42.75 ± 0.48 | 38.75 ± 1.25 | 16.43 ± 0.08 | 3.83 ± 0.05 |
| | **MLESV** | **1000** | 131.00 ± 0.91* | 45.50 ± 1.56* | 77.25 ± 1.49 | 41.50 ± 1.19 | 42.25 ± 0.5* | 16.93 ± 0.06 | 4.08 ± 0.14 |

Values are expressed as Mean ± SEM; n=10

D/water = Distilled water

MLESV= Methanol Leaf Extract of *Securinegavirosa*

*Significantly different from control at P□0.05

AST = Aspartate Aminotransferase
ALT = Alanine Aminotransferase
Tpr = Total Protein
Alb = Albumin
ALP = Alkaline Phosphatase
DBil = Direct Bilirubin
InBil = Indirect Bilirubin

### 4.4.7 Effect of methanol leafextract of *Securinega virosa* on Lipid Profile of Wistar rats after oral administration for 28 days

There was no significant ($p$ 0.05) difference in the lipid profile (total cholesterol, triglicerides, low density lipoprotein, and high density lipoprotein) in both male and female rats when compared with control (Table 4.11).

**Table 4.11: Effect of Methanol Leaf Extract of *Securinega virosa* on Lipid Profile of Wistar Rats After Oral Administration for 28 Days**

| | Treatment | (mg/kg) | TCHOL(mg/dl) | TRIG(mg/dl) | HDL(mg/dl) | LDL(mg/dl) |
|---|---|---|---|---|---|---|
| **Male** | **D/Water** | **2 (ml/kg)** | 17.85 ± 0.41 | 1.88 ± 0.03 | 1.98 ± 0.01 | 8.15 ± 0.10 |
| | **MLESV** | **250** | 16.80 ± 0.12 | 1.73 ± 0.01 | 2.06 ± 0.01 | 7.80 ± 0.29 |
| | **MLESV** | **500** | 17.08 ± 0.06 | 1.57 ± 0.05 | 2.12 ± 0.01 | 8.13 ± 0.24 |
| | **MLESV** | **1000** | 17.73 ± 0.15 | 1.69 ± 0.12 | 2.15 ± 0.01 | 8.13 ± 0.20 |
| **Female** | **D/Water** | **2 (ml/kg)** | 16.00 ± 0.09 | 1.78 ± 0.03 | 1.64 ± 0.03 | 13.30 ±0.22 |
| | **MLESV** | **250** | 15.98 ± 0.08 | 1.68 ± 0.03 | 1.76 ± 0.01 | 13.05 ± 0.13 |
| | **MLESV** | **500** | 15.90 ± 0.23 | 1.77 ± 0.01 | 1.88 ± 0.01 | 13.28 ± 0.19 |
| | **MLESV** | **1000** | 15.48 ± 0.43 | 1.87 ± 0.02 | 2.00 ± 0.01 | 12.45 ± 0.27 |

Values are expressed as Mean ± SEM; n=10

D/water = Distilled water

MLESV= Methanol Leaf Extract of *Securinegavirosa*

TCHOL = Total Cholesterol
TRIG = Triglycerides
HDL = High Density Lipoprotein
LDL = Low Density Lipoprotein

## 4.5 Histopathological Findings on Some Organs after Oral Administration for 28 days with Methanol Leaf Extract of *Securinega virosa*.

There were no gross pathological changes observed inthe animals and their organs at the end of the experiment. Histopathological analysis of the various organs is as described below.

### 4.5.1 Histopathological findings on the liver afteroral administration for 28 days with methanol leaf extract of *Securinega virosa*

Photomicrograph of the liver sections of both male and female control animals showed normal hepatic architecture and normal liver lobular structure with well-preserved cytoplasm. Hepatic cell toxicity characterized by centrilobular necrosis, peri-portal hepatocytic vacuolation and inflammatory changes were observed starting from the female group treated with 250, 500 and 1000mg/kg body weight. In male rats, hepatic toxicity was seen at doses of 500 and 1000mg/kg body weight (Plate III and IV).

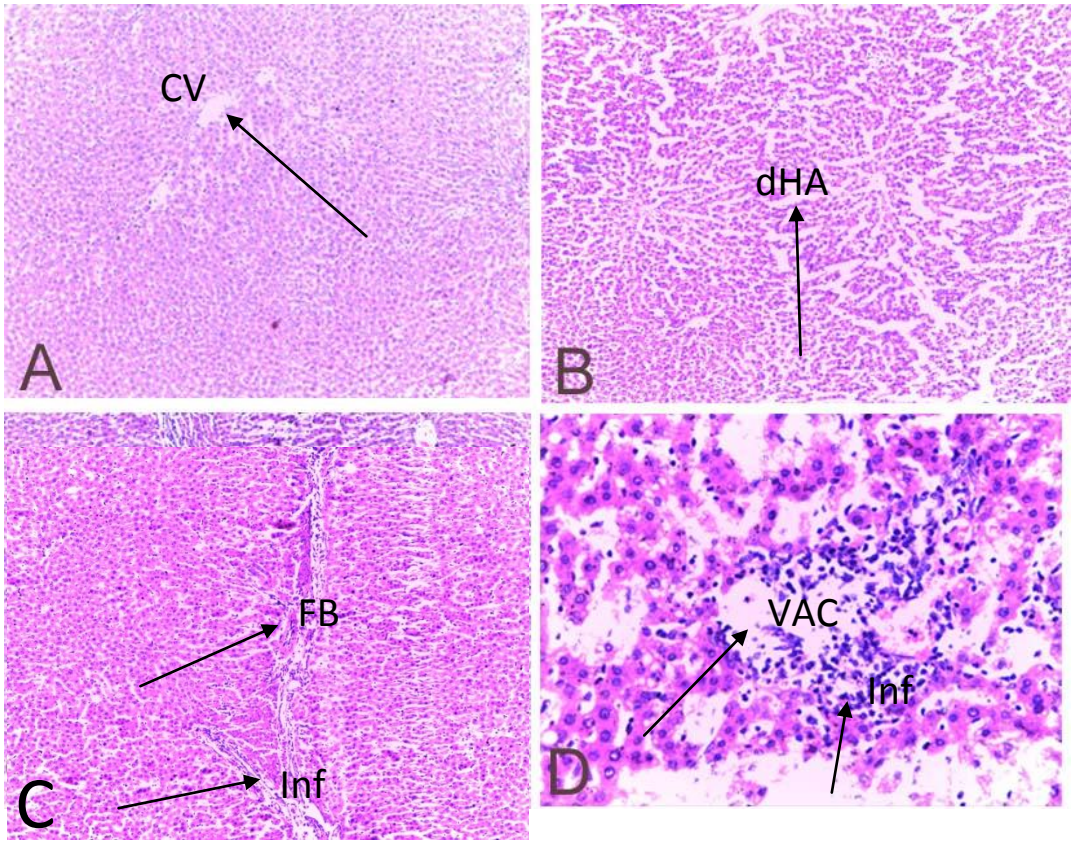



**Plate III: Effect of methanol leaf extract of *Securinega virosa* on liver histomorphology in femalerats after oral administration for 28 days**

**A=Control; Arrow showing normal central vein(CV) (H and E, ×100)**

**B=250mg/kg extract,Arrow showing distorted hepatic architecture (dHA) and central vein (H and E, ×100)**

**C=500mg/kg extract, centrilobular necrosis, fibrosis (FB) and inflammatory changes (Inf) (H and E,× 100)**

**D=1000mg/kg extract, peri-portal hepatocytic vacuolation (VAC), centrilobular necrosis and scattered inflammatory changes (Inf), (H and E, ×400)**

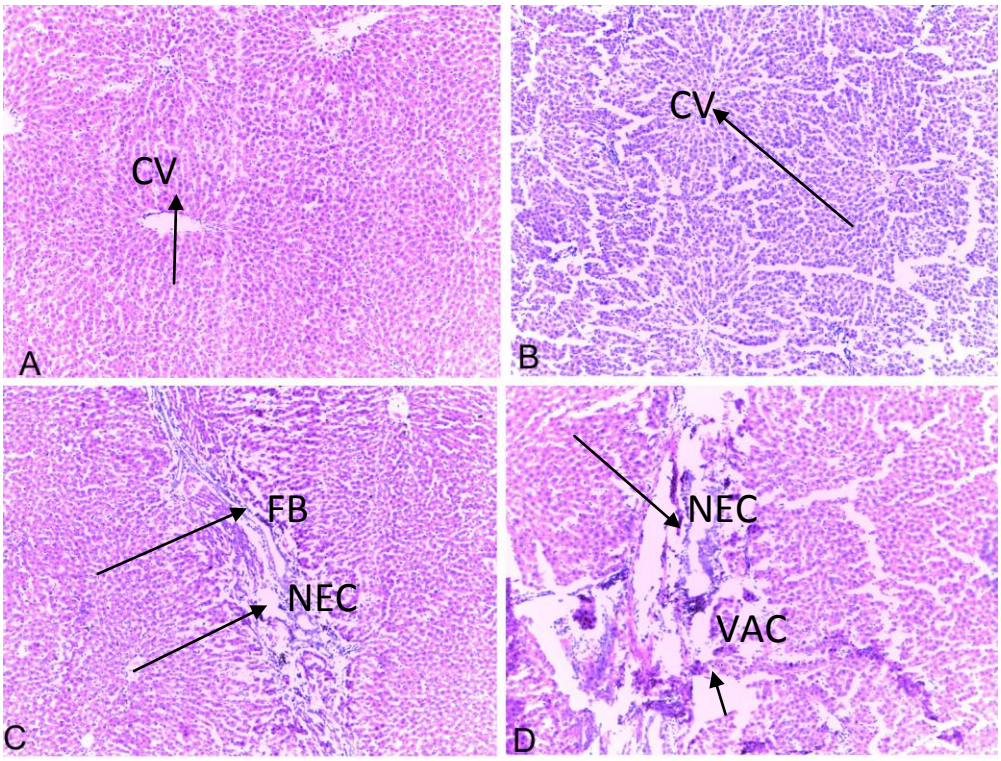



**Plate IV:Effect of methanol leaf extract of *Securinega virosa* on liver histomorphology in malerats after oral administration for28 days**

**A=Control,Arrow shows normal central vein (CV)**

**B=250mg/kg extract, apparently normal central vein (CV) and hepatic architecture**

**C=500mg/kg extract, centrilobular necrosis (NEC), fibrosis (FB) and inflammatory changes**

**D=1000mg/kg extract, peri-portal hepatocytic vacuolation (VAC), centrilobular necrosis (NEC) and scattered inflammatory changes**

**(H and E, ×100)**

### 4.5.2 Histopathological findings on the kidney after 28 days oral administration of methanol leaf extract of *Securinega virosa*

Histopathological sections of the kidneys of both male and female rats in the groups treated with 500mg/kg body weight of the extract showed features of glomerular damage, focal glomerular atrophy, and vascular congestion in addition to fibrosis observed in both male and female groups treated with 1000mg/kg body weight of the extract. The glomerular damage was not observed in the groups treated with 250mg/kg body weight and the cellular architecture was normal (Plate V and VI).

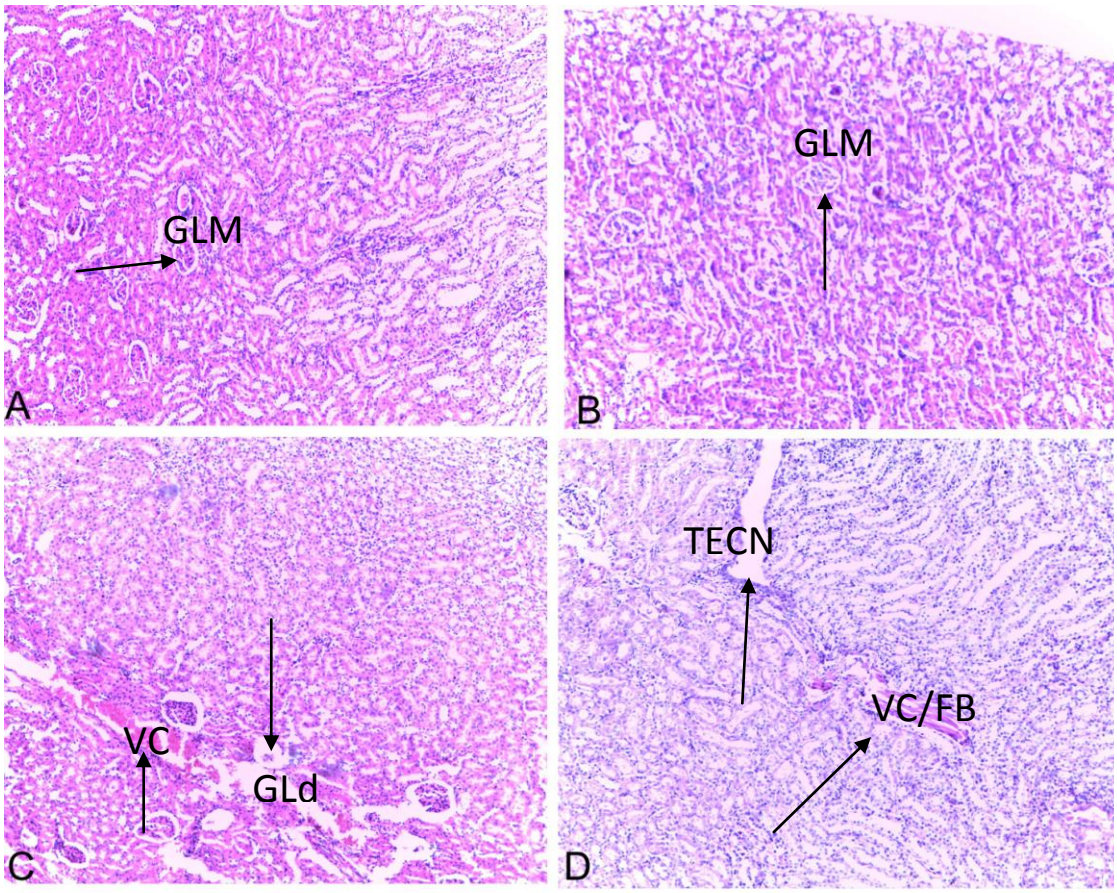



**Plate V:Effect of methanol leaf extract of *Securinega virosa* on kidney histomorphology in femalerats after 28 days oral administration**

**A=Control, normal glomerulus (GLM)**

**B=250mg/kg extract, apparently normal glomerulus (GLM)**

**C=500mg/kg extract,glomerular damage and atrophy (GLd) , vascular congestion (VC)**

**D=1000mg/kg extract,glomerular damage, tubular epithelial cell necrosis (TECN) vascular Congestion and fibrosis (VC/FB)**

**(H and E, ×100)**

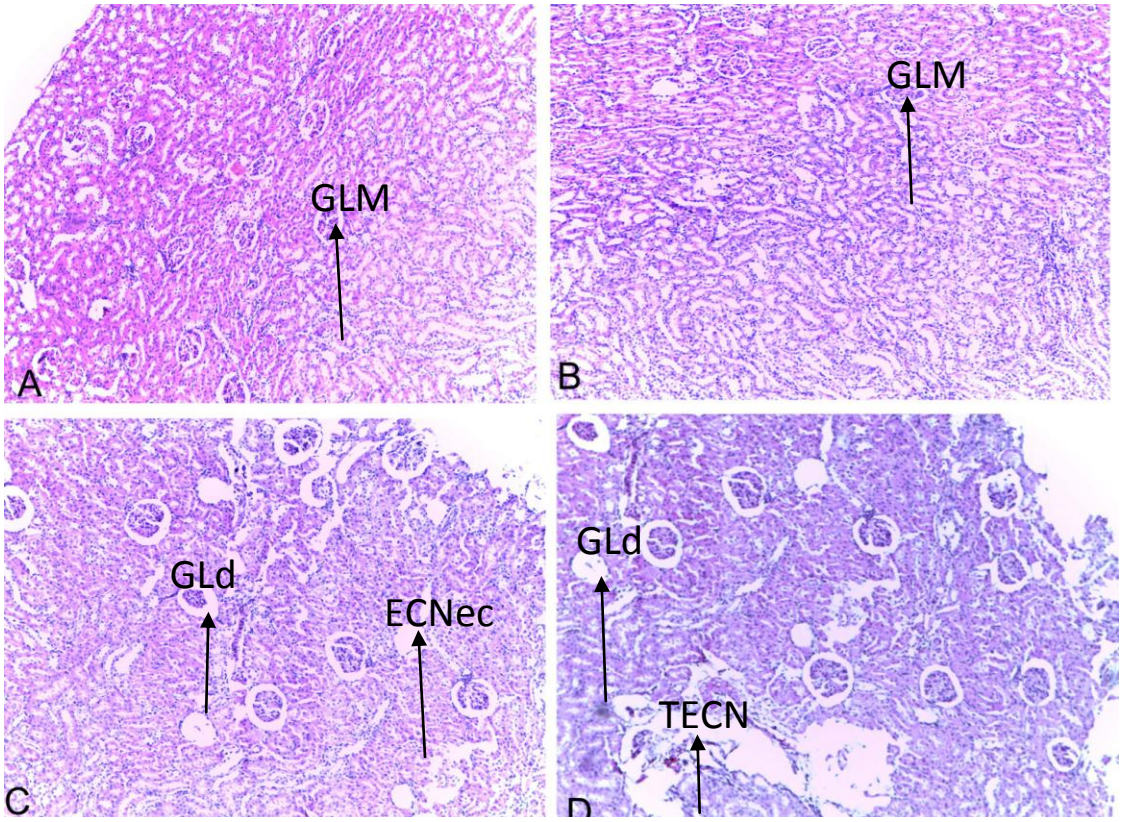



**Plate VI:Effect of methanol leaf extract of *Securinega virosa* on kidney histomorphology in malerats after 28 days oral administration**

**A=Control, normal glomerulus (GLM)**

**B=250mg/kg extract, apparently normal glomerulus (GLM)**

**C=500mg/kg extract, glomerular damage (GLd) and epithelial cell necrosis (ECNec)**

**D=1000mg/kg extract,glomerular damage (GLd), tubular epithelial cell necrosis TECN), (H and E, ×100)**

### 4.5.3 Histopathological findings on the spleen after 28 days oral administration of methanol leaf extract of *Securinega virosa*

Splenic congestion was observed in the histopathological sections of the spleen at all dose levels in both male and female rats. The histopathological section of the spleen in the control group showed normal splenic architecture with the white pulp containing lymphoid nodules and the red pulp containing blood filled sinusoids (Plate VII and VIII).

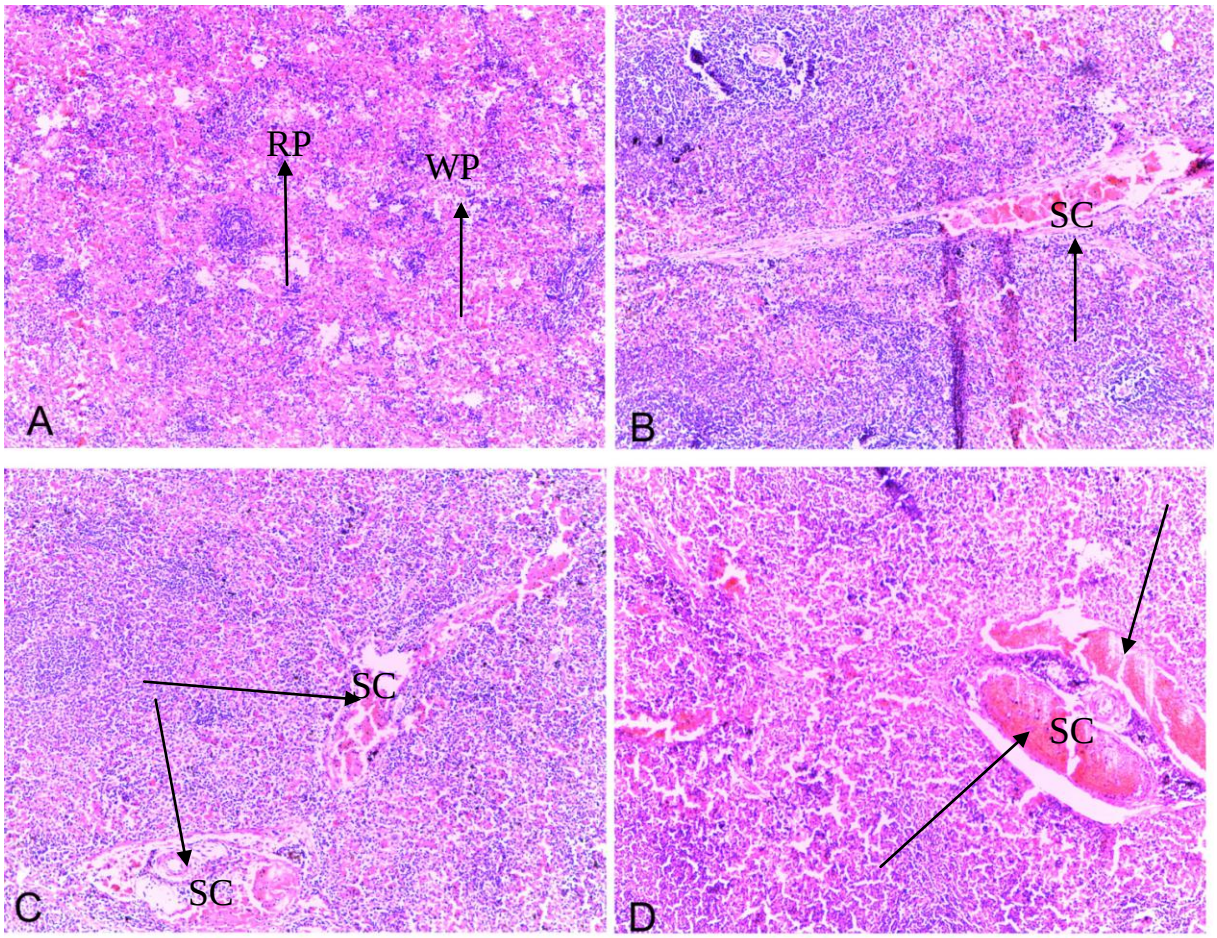



**Plate VII:Effect of methanol leaf extract of *Securinega virosa* on spleenhistomorphology in femalerats after 28 days oral administration**

**A=Control, normal splenic architecture showing red pulp (RP) and white pulp (WP)**

**B=250mg/kg extract, splenic congestion (SC)**

**C=500mg/kg extract, splenic congestion (SC)**

**D=1000mg/kg extract, splenic congestion (SC)**

**(H and E, ×100)**

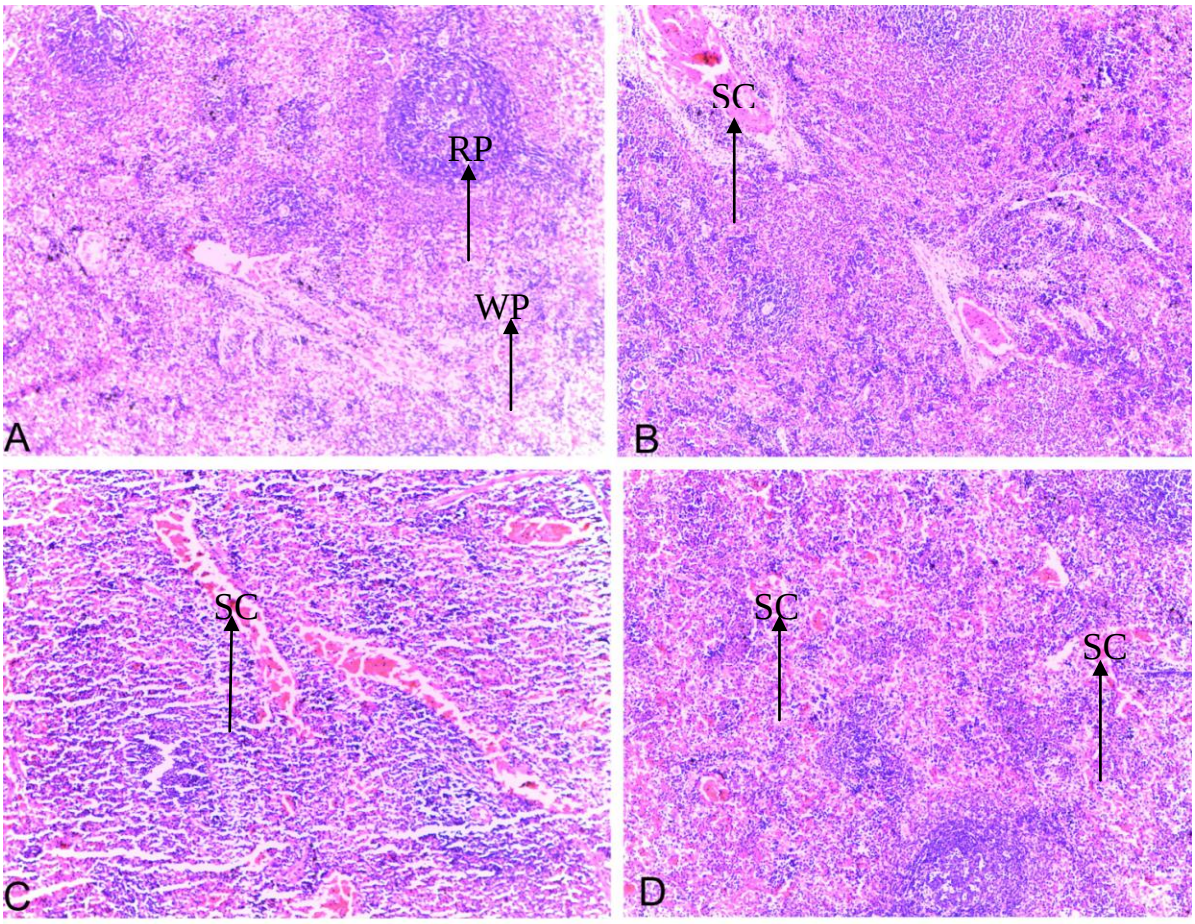



**Plate VIII:Effect of methanol leaf extract of *Securinega virosa* on spleen histomorphology infemalerats after 28 days oral administration**

**A=Control, normal splenic architecture showing red pulp (RP) and white pulp (WP)**

**B=250mg/kg extract, splenic congestion (SC)**

**C=500mg/kg extract, splenic congestion (SC)**

**D=1000mg/kg extract, splenic congestion (SC)**

**(H and E, ×100)**

### 4.5.4 Histopathological findings on the lungs after 28 days oral administration of methanol leaf extract of *Securinega virosa*

The histopathological sections of the lungs showed diffused areas of congestion and inflammation of the alveoli and terminal bronchioles in both male and female rats treated with 500and 1000mg/kg body weight of the extract. There was however unremarkable changes in the histopathological sections of the lungs in the control groups and the rats treated with 250mg/kg body weight of the extract (Plate IX and X).

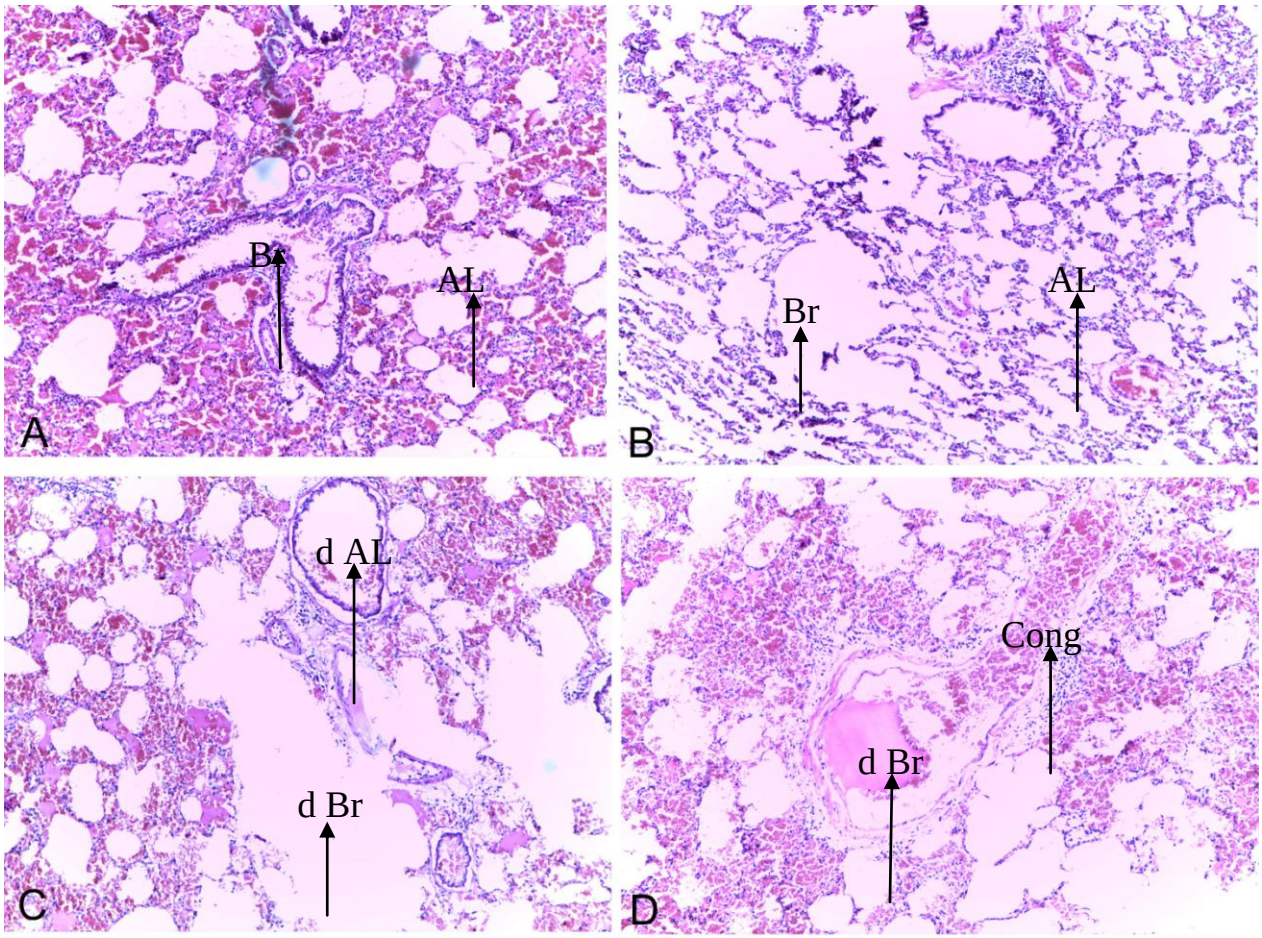



**Plate IX:Effect of methanol leaf extract of *Securinega virosa* on lungs histomorphology in femalerats after 28 days oral administration**

**A=Control, normal lungs parenchyma showing bronchiole (Br) and alveoli (AL)**

**B=250mg/kg extract, normal lungs parenchyma showing bronchiole (Br) and alveoli (AL)**

**C=500mg/kg extract, damage to the alveoli (d AL) and terminal bronchiole (d Br)**

**D=1000mg/kg extract,diffused areas of congestion (cong) inflammation and damage to the alveoli and terminal bronchioles (d Br)**

**(H and E,×100)**

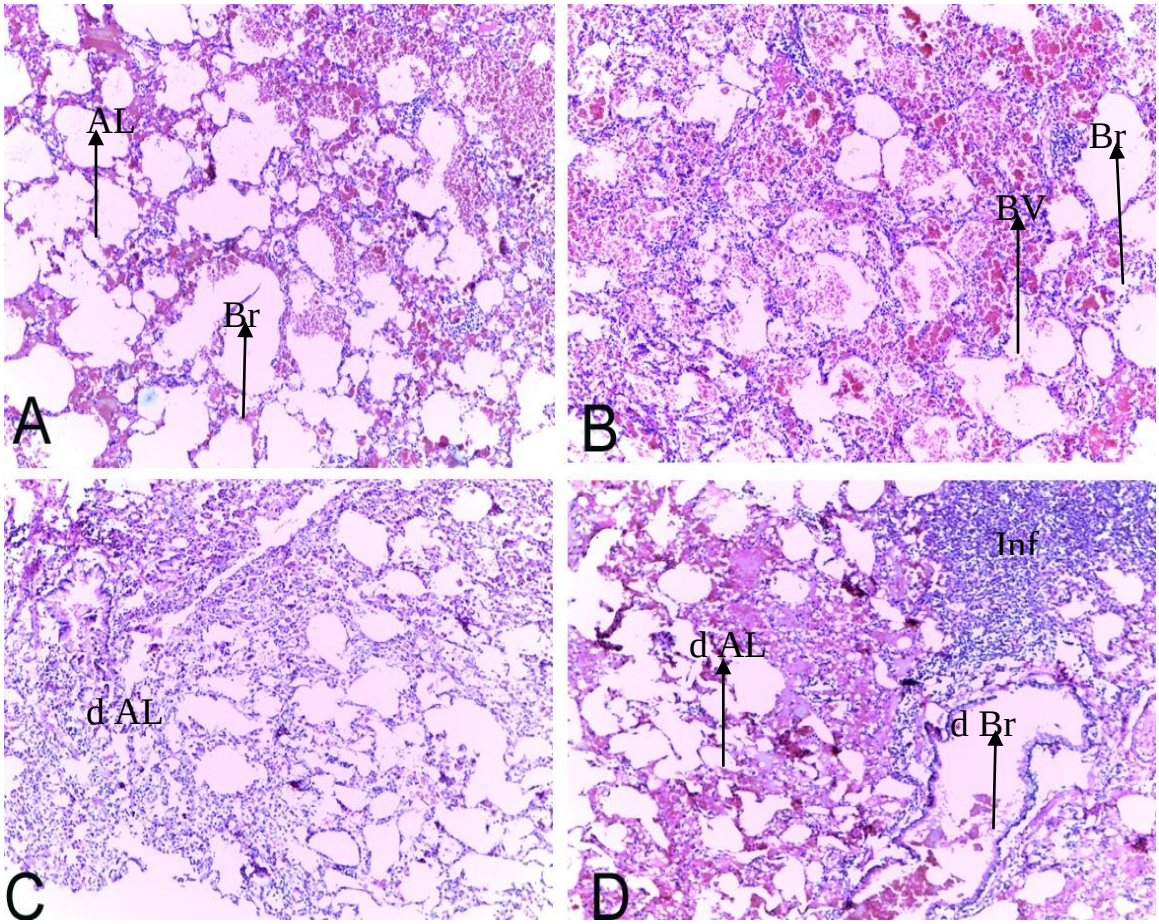



**Plate X:Effect of methanol leaf extract of *Securinega virosa* on lungs histomorphology in malerats after 28 days oral administration**

**A=Control, normal lungs parenchyma showing bronchiole (Br) and alveoli (AL)**

**B=250mg/kg extract, normal lungs parenchyma with blood vessels (BV) and bronchioles**

**C=500mg/kg extract, damage to the alveoli (d AL) and terminal bronchiole (d Br)**

**D=1000mg/kg extract,diffused areas of congestion (Cong) inflammation (Inf) and damage to the alveoli (d AL) and terminal bronchioles (d Br)**

**(H and E, ×100)**

### 4.5.5 Histopathological findings on the heart after 28 days oral administration of methanolleaf extract of *Securinega virosa*

The histopathological sections of the heart showed areas of myocardial haemorrhage in female rats treated with 500mg/kg body weight of the extract. Haemorrhage with thickening of the myocardium of the female rats treated with 1000mg/kg body weight. The histopathological sections of the heart in female rats treated with 250mg/kg body weight did not show obvious architectural changes that were different from the control. The histopathological sections of the heart in male rats did not show obvious architectural changes that were different from the control at all dose levels (Plate XI and XII).

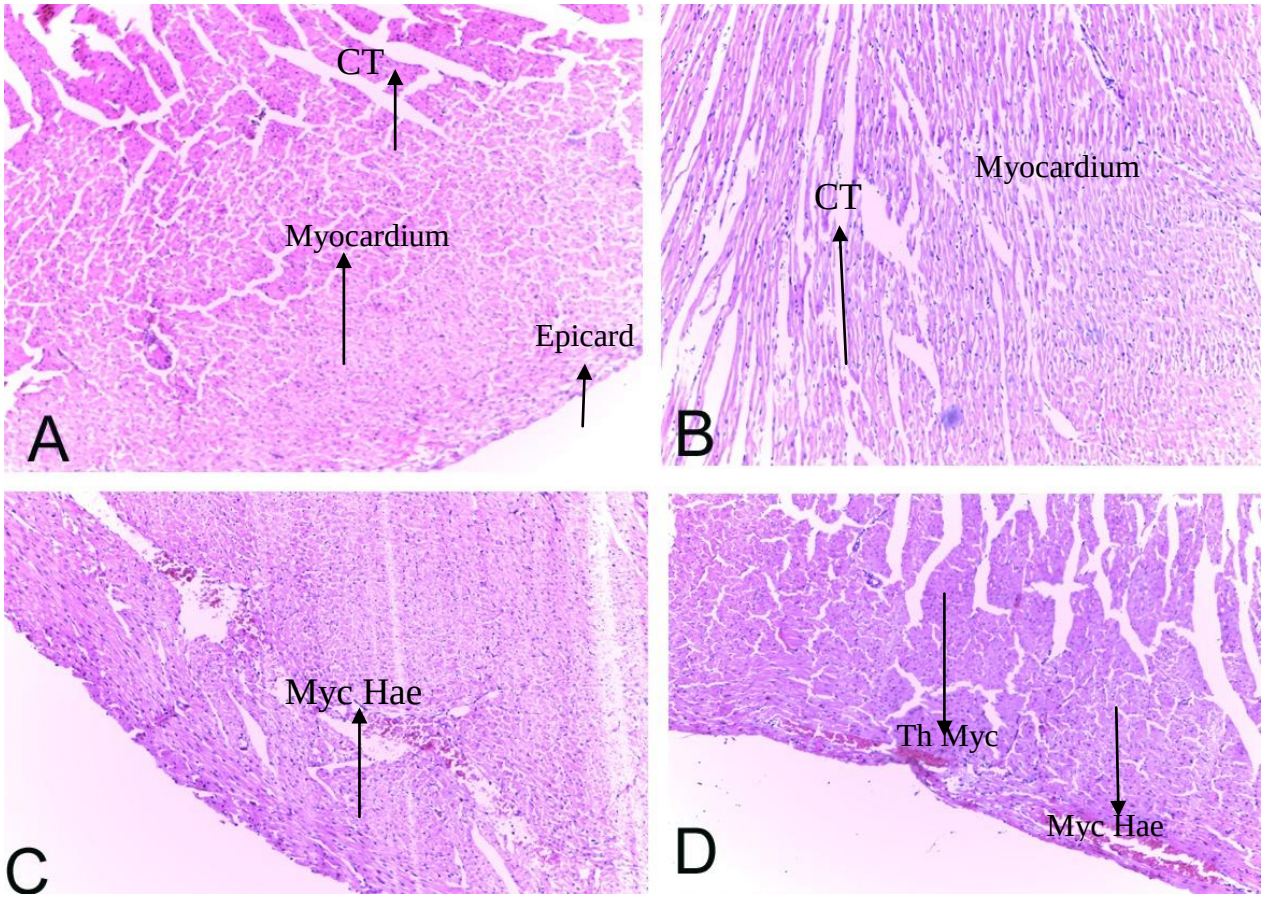



**Plate XI:Effect of methanol leaf extract of *Securinega virosa* on heart histomorphology in femalerats after 28 days oral administration**

**A=Control, normal myocardium, epicardium (Epicard) and Connective tissue (CT)**

**B=250mg/kg extract, normal myocardium and connective tissue (CT)**

**C=500mg/kg extract,arrow showing myocardial haemorrhage (Myc Hae)**

**D=1000mg/kg extract, haemorrhage (Myc Hae) with thickening of myocardium (Th Myc)**

**(H and E, ×100)**

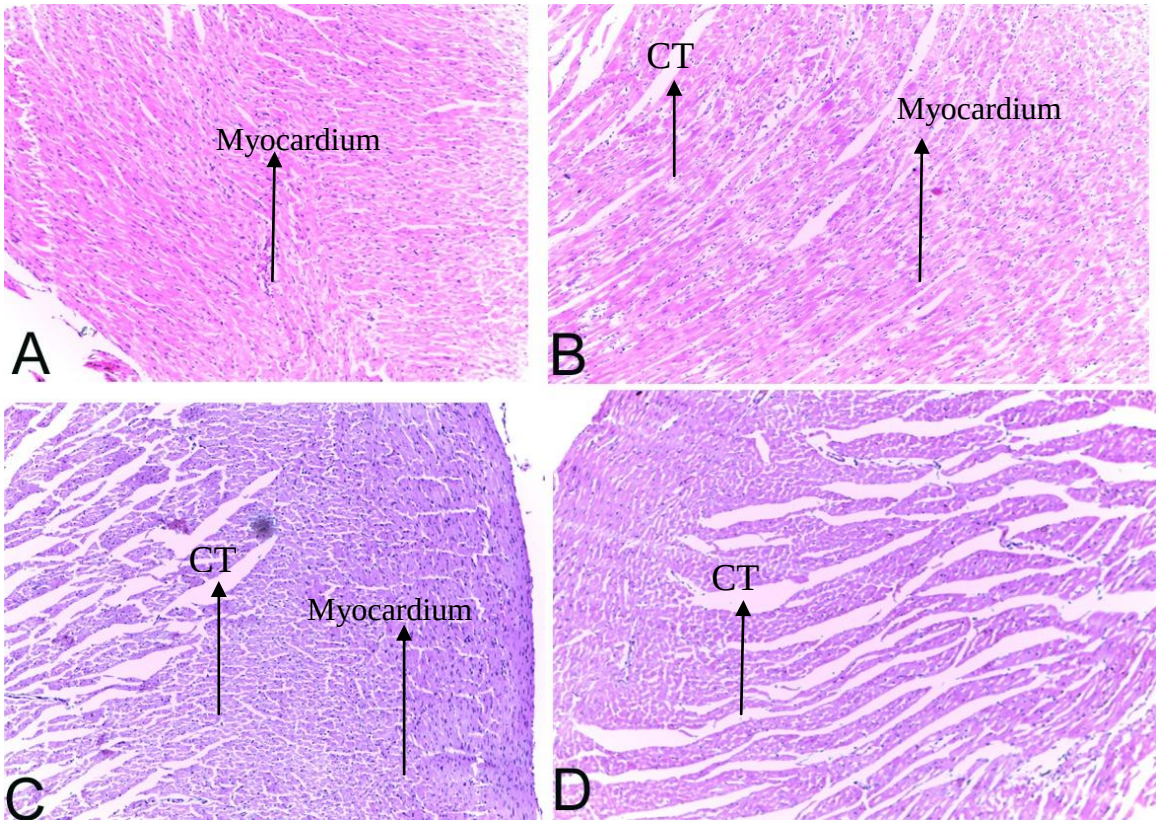



**Plate XII:Effect of methanol leaf extract of *Securinega virosa* on hearthistomorphology in malerats after 28 days oral administration**

**A=Control, arrow showing normal myocardium**

**B=250mg/kg extract,arrow showing normal myocardium and connective tissue (CT)**

**C=500mg/kg extract,arrow showing normal myocardium and connective tissue (CT)**

**D=1000mg/kg extract, arrow showing normal myocardium and connective tissue (CT)**

**(H and E,×100)**

### 4.5.6 Histopathological findings on the brain after 28 days oral administration of methanol leaf extract of *Securinega virosa*

The histopathological sections of the brain showed shrinking of the cellular layers and pyknotic cells, distortortion and clumping of cells in the granular layers of the cerebellum while diffused areas of degeneration of nerve fibres and vacuolation of the cerebral cortexwere also noted in male and female rats treated with 250 500and 1000mg/kg body weight of the extract, The histopathological sections of the brainin male and female rats in control group showed unremarkable brain tissue with well defined molecular, cellular and granular layers of the cerebellum and pyramidal neurons in the cerebrum interspersed with numerous glial cells (Plate XIII, XIV, XV, and XVI).

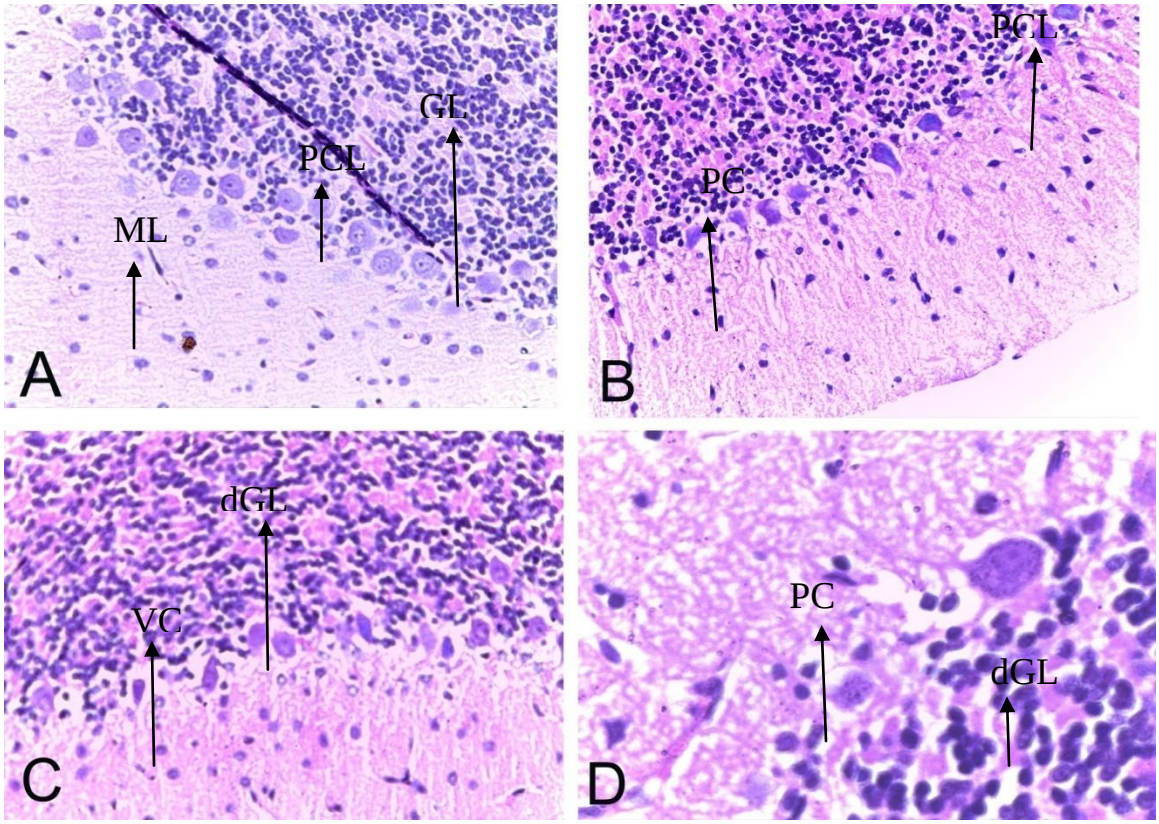



**Plate XIII:Effect of methanol leaf extract of *Securinega virosa* on brain (cerebellum) histomorphology in femalerats after 28 days oral administration**

**A=Control, normal cerebellar molecular layer (ML), purkinje cell layer (PCL) and granular layer (GL)(H and E, ×100);**

**B=250mg/kg extract; mild shrinking of the cellular layer (PCL) and pyknotic cells (PC) (H and E, ×100)**

**C=500mg/kg extract,distortion of the granular layer (dGL) and vacuolation of the purkinje cells (VC)(H and E, ×100)**

**D=1000mg/kg extract, pyknotic cells (PC) and marked distortion of the granular cell layers (dGL)(H and E, ×400)**

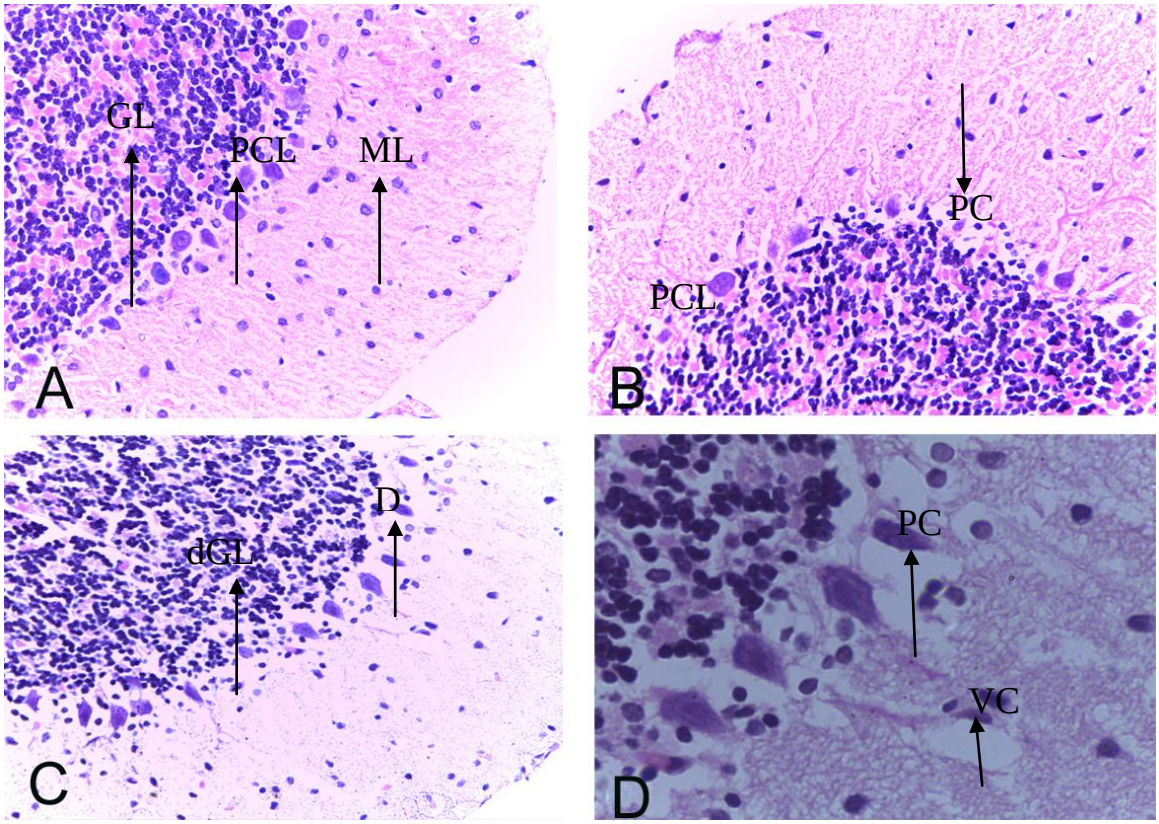



**Plate XIV:Effect of methanol leaf extract of *Securinega virosa* on brain (cerebellum) histomorphology in malerats after 28 days oral administration**

**A=Control, normal cerebellar molecular layer (ML), purkinje cell layer (PCL) and granular layer (GL) (H and E, ×100)**

**B=250mg/kg extract, mild shrinking of thepurkinje cell layer (PCL) and pyknotic cells (PC) (H and E, ×100)**

**C=500mg/kg extract, distortion of the granular and degeneration of the purkinje cell layers(D) (H and E, ×100)**

**D=1000mg/kg extract, pyknotic cells (PC) and vacuolated molecular cells (VC)(H and E, ×400)**

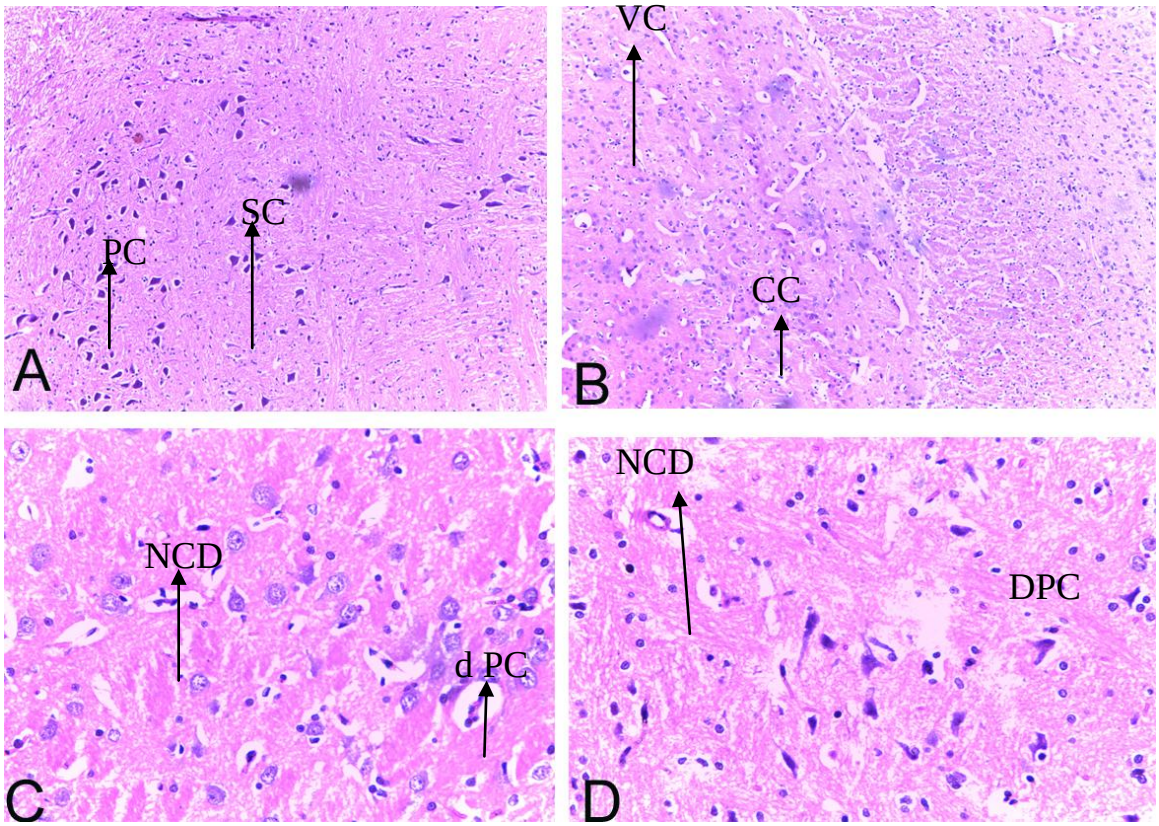



**Plate XV:Effect of methanol leaf extract of *Securinega virosa* on brain (cerebrum) histomorphology in femalerats after 28 days oral administration**

**A=Control, normal cerebral architecturewith arrows showing pyramidal cell (PC) and stellate cell (SC) (H and E, ×40)**

**B=250mg/kg extract,vacuolation (VC) and clumping of cells (CC) (H and E, ×100)**

**C=500mg/kg extract,diffused areas of neuronal cell damage (NCD) and degenerated pyramidal cells (DPC) (H and E, ×100)**

**D=1000mg/kg extract,diffused areas of degeneration of nerve fibres (NCD) and degenerated pyramidal cells (DPC) (H and E, ×100)**

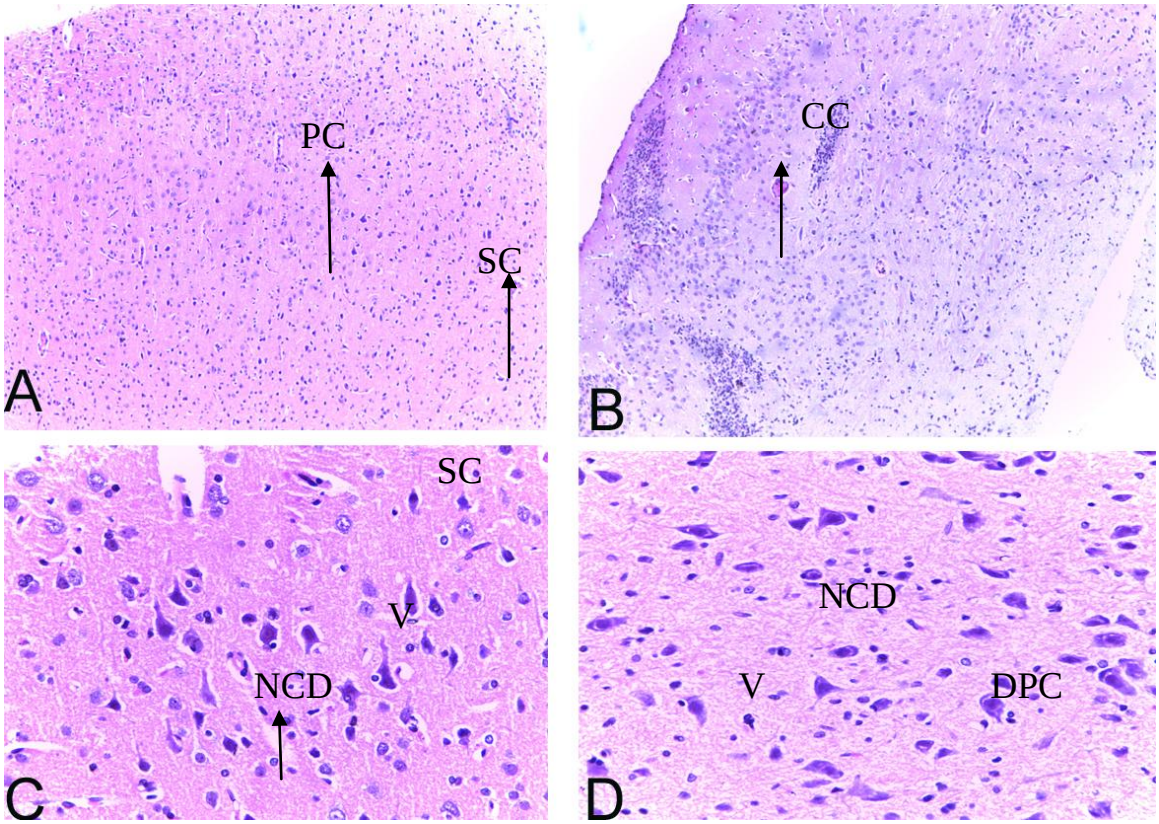



**Plate XVI:Effect of methanol leaf extract of *Securinega virosa* on brain (cerebrum) histomorphology in malerats after 28 days oral administration**

**A=Control, normal cerebral architectureshowing small pyramidal cells (PC) and stellate cells (SC) (H and E, ×40)**

**B=250mg/kg extract,clumping of cells (CC) (H and E, ×40)**

**C=500mg/kg extract, diffused areas of neuronal cell damage (NCD)and stellate cells (SC) (H and E, ×100)**

**D=1000mg/kg extract,diffused areas of degeneration of nerve cells (NCD) and degenerated pyramidal cells (DPC) (H and E, ×100)**

### 4.5.6 Histopathological findings on the stomach after 28 days oral administration of methanol leaf extract of *Securinega virosa*

The histopathological sections of the stomach in both male and female rats at all doses tested showed normal findings at the mucosa, muscularis and serosa that were not different from the control (Plate XVII and XVIII).

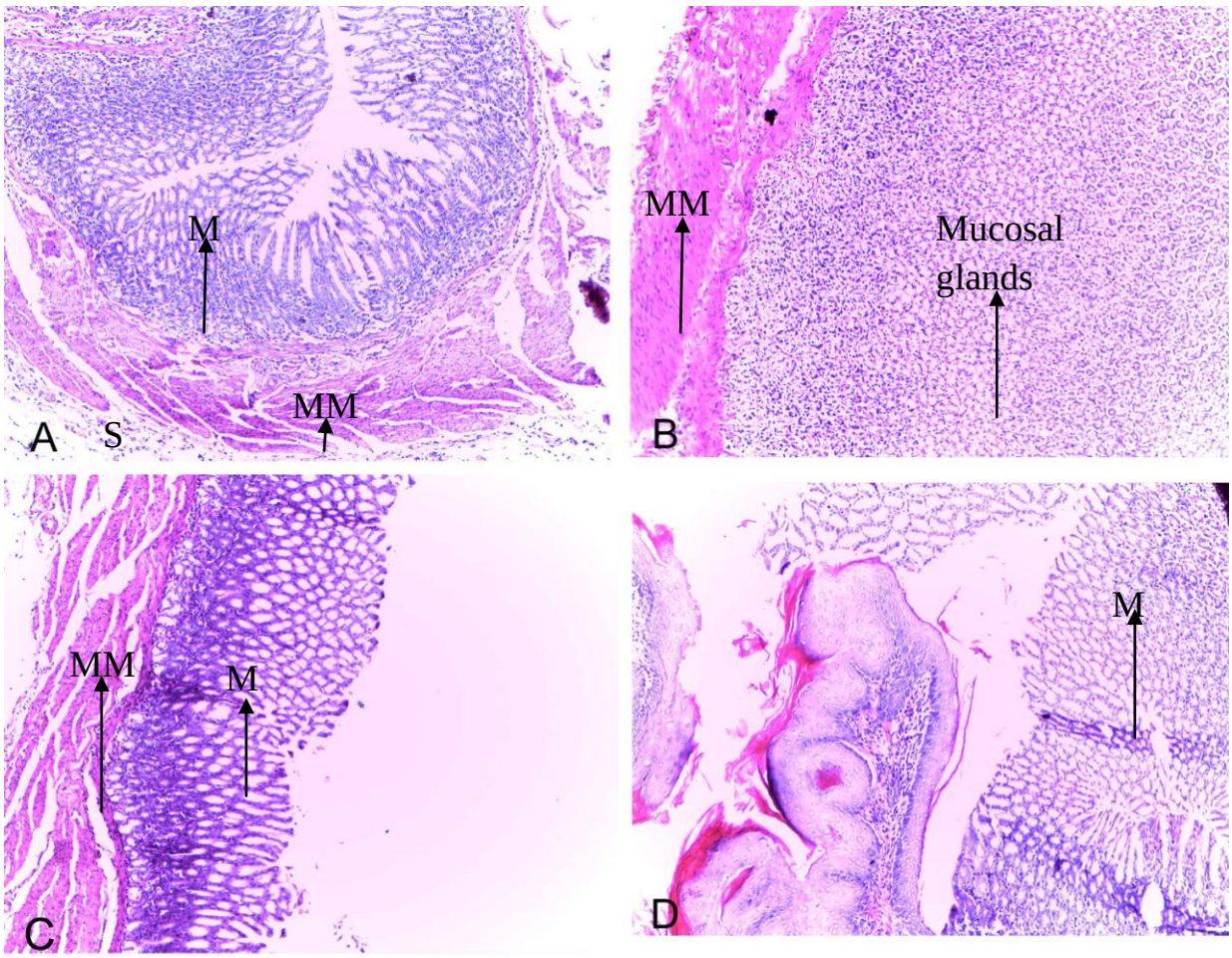



**Plate XVII:Effect of methanol leaf extract of *Securinega virosa* on stomach histomorphology in femalerats after 28 days oral administration**

**A=Control, normal mucosal lining (M), muscularis mucosa (MM) and serosa (S)**

**B=250mg/kg extract, normal mucosal glands and muscularis mucosa (M)**

**C=500mg/kg extract, normal mucosa (M) and muscularis mucosa (MM)**

**D=1000mg/kg extract, normal mucosal lining (M)**

**(H and E, ×100)**

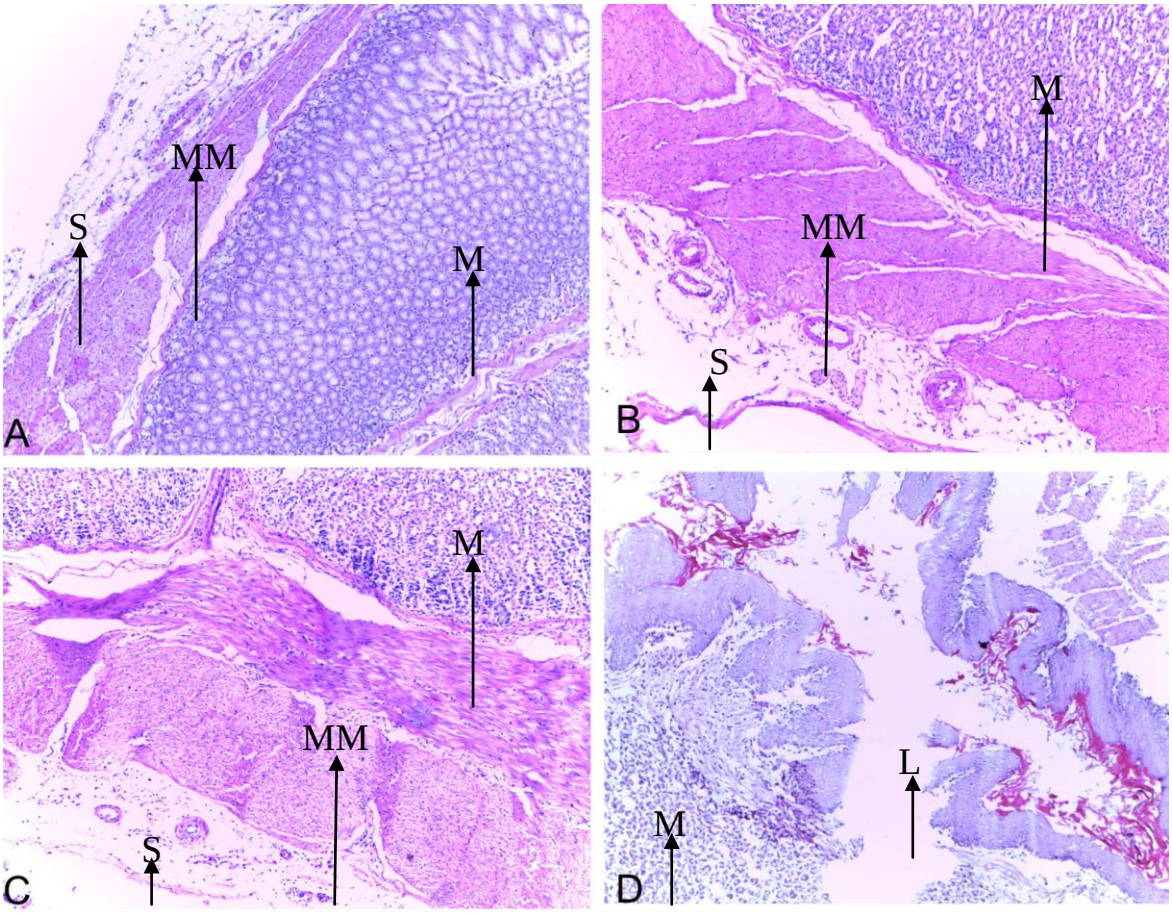



**Plate XVIII:Effect of methanol leaf extract of *Securinega virosa* on stomach histomorphology in malerats after 28 days oral administration**

**A=Control, normal mucosal lining (M), muscularis mucosa (MM) and serosa (S)**

**B=250mg/kg extract, normal mucosa (M), muscularis mucosa (MM) and serosa (S)**

**C=500mg/kg extract, normal mucosa (M), muscularis mucosa (MM) and serosa (S)**

**D=1000mg/kg extract, normal mucosal lining (M) and lumen (L)**

**(H and E ×100)**

### 4.5.6 Histopathological findings on the uterus after 28 days oral administration of methanolleaf extract of *Securinega virosa*

The histopathological sections of the uterus in female rats showed normal findings with the endometrium showing several endometrial glands that were not different from the control at all the dosestested (Plate XIX)

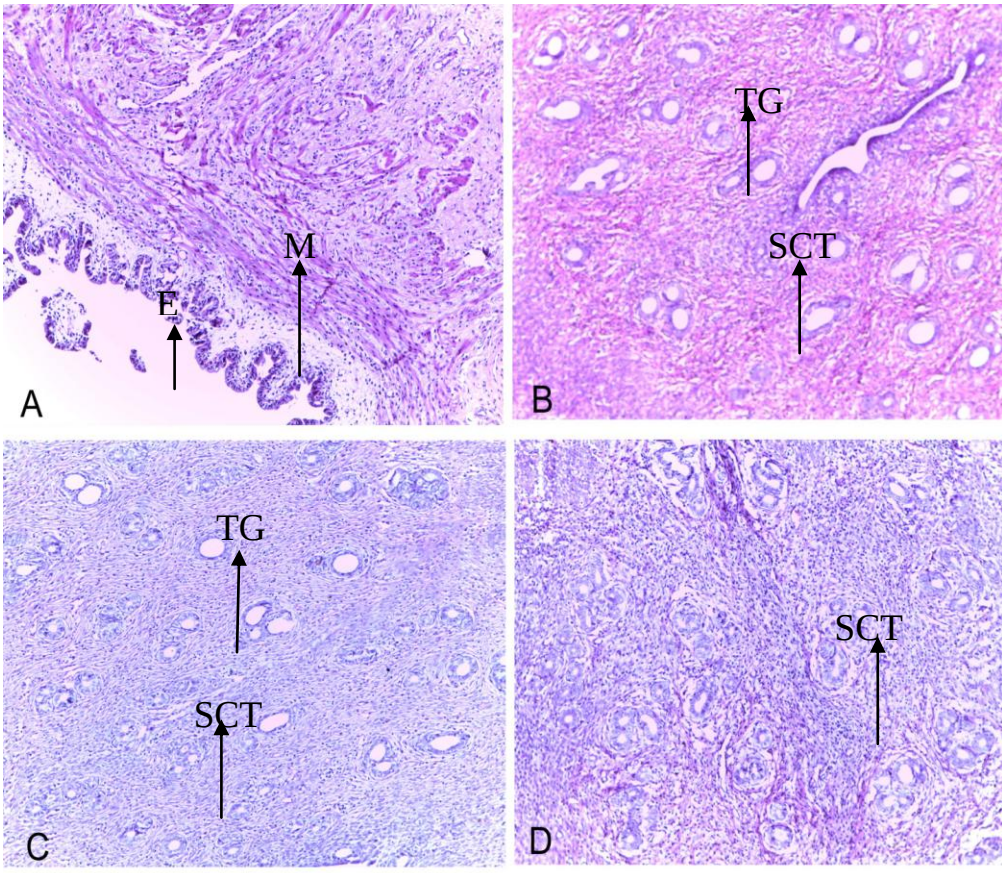



**Plate XIX:Effect of methanol leaf extract of *Securinega virosa* on uterus histomorphology in rats after 28 days oral administration**

**A=Control, normal endometrium (E) and myometrium (M);B=250mg/kg extract,normal endometrial tubular glands (TG) and stromal connective tissue (SCT);C=500mg/kgextract,normal endometrial glands (TG); D=1000mg/kg extract, normal stromal connective tissue (SCT)**

**(H and E, ×100)**

### 4.5.7 Histopathological findings on the ovaries after 28 days oral administration of methanol leaf extract of *Securinega virosa*

The histopathological sections of the ovaries in female rats showed normal findings with ovarian follicles at various stages of development that were not different from the control at all dosestested (Plate XX).

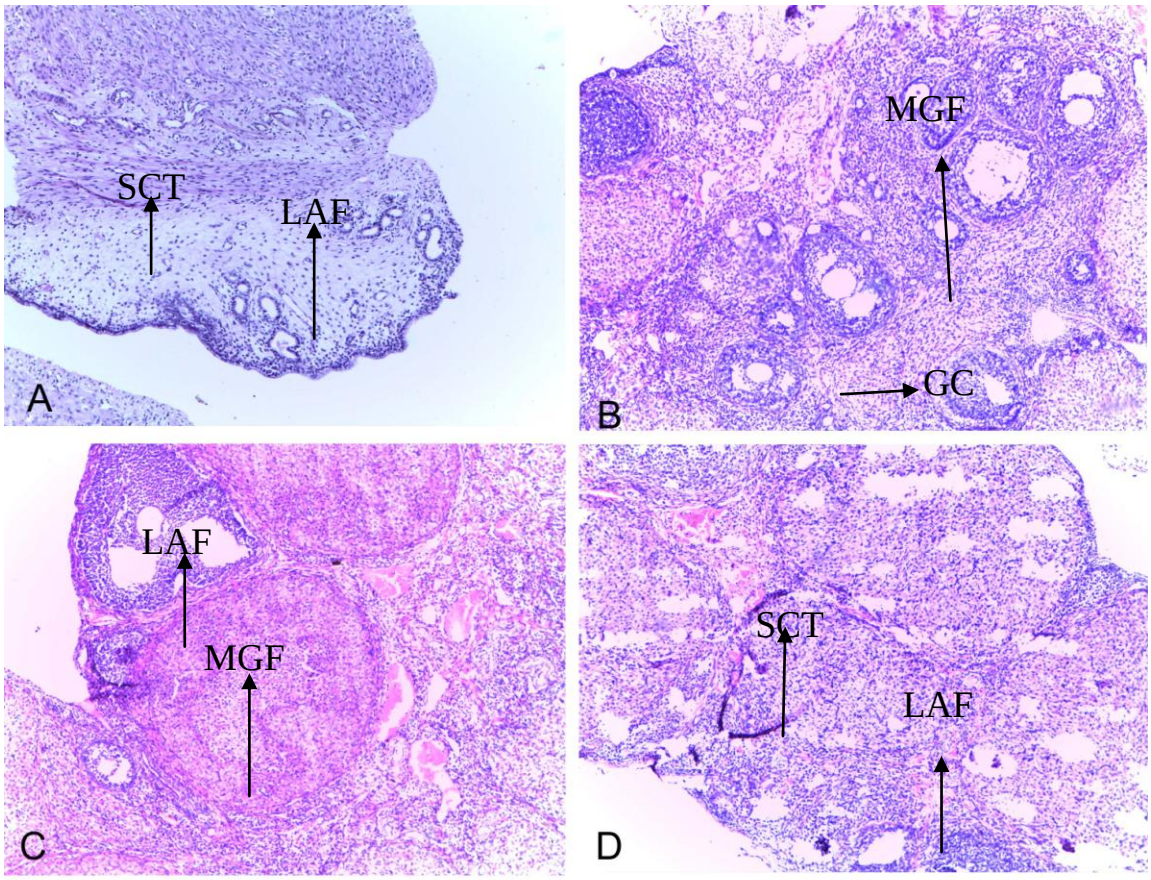



**Plate XX:Effect of methanol leaf extract of *Securinega virosa* on ovaries histomorphology in rats after 28 days oral administration**

**A=Control, normal stromal connective tissue (SCT) and late atretic follicles (LAF); B=250mg/kg extract, matured graffian follicle (MGF) and granulose cells (GC) C=500mg/kg extract, matured graffian follicles (MGF); D=1000mg/kg extract, stromal connective tissue (SCT) and late atretic follicle (LAF) (H and E, ×100)**

### 4.5.7 Histopathological findings on the testes after 28 days oral administration of methanol leaf extract of *Securinega virosa*

The histopathological sections of the testes in male rats showed normal findings with normal testicular tissue consisting of seminiferous tubules lined by spermatogenic cells at various stages of development that were not different from the control at all dosestested (Plate XXI).

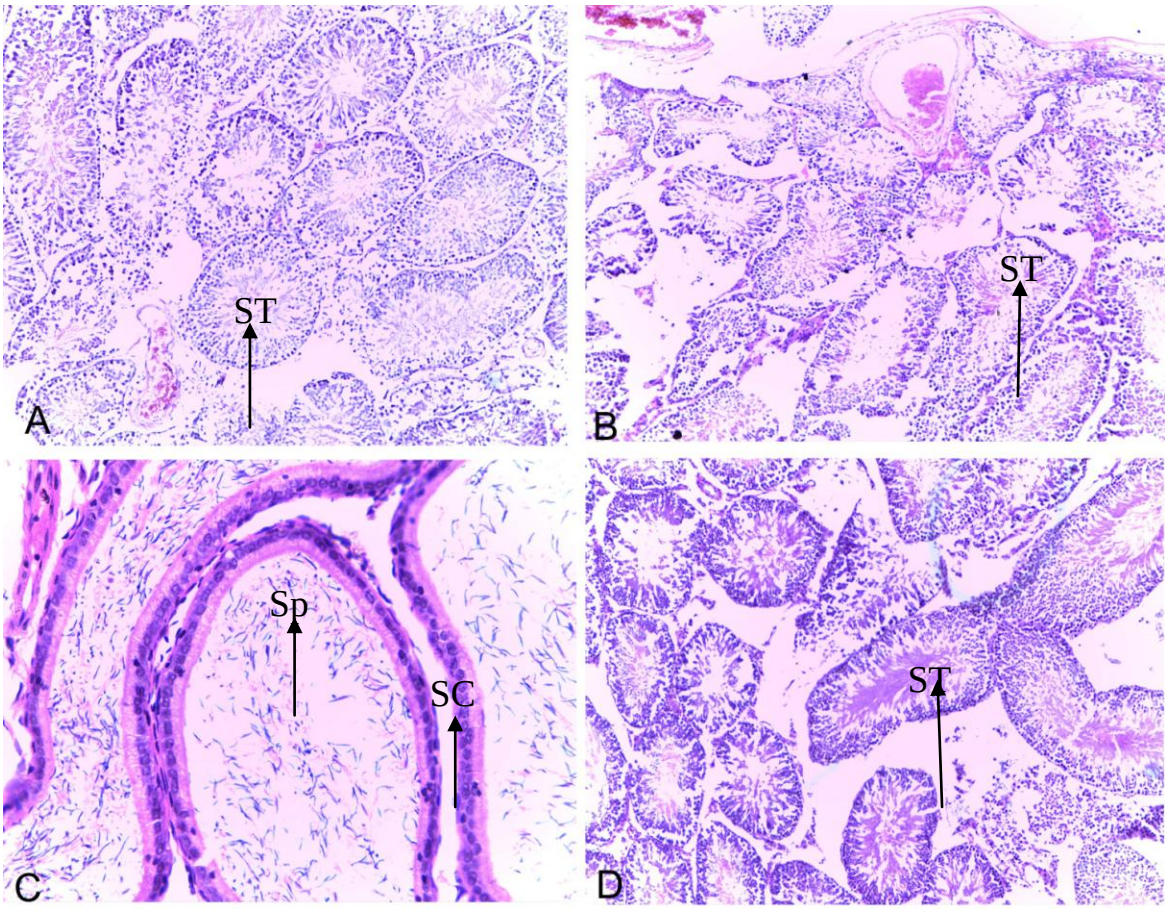



**Plate XXI:Effect of methanol leaf extract of *Securinega virosa* on testeshistomorphology in rats after 28 days oral administration**

**A=Control, normal testicular tissue with seminiferous tubules (ST)**

**B=250mg/kg extract, normal seminiferous tubules (ST)**

**C=500mg/kg extract,normal seminiferous tubules with spermatozoa (Sp) and spermatogenic cells (SC)**

**D=1000mg/kg extract, normal seminiferous tubules (ST)**

**(H and E, ×100)**

# CHAPTER FIVE

## 5.0 DISCUSSION

The need to evaluate the toxicity profile of *Securinega virosa* leaf extract was prompted by its widespread use in the management of various disorders, and has been generally regarded as a plant that "cures all" (Neuwinger, 1996;Magaji *et al*., 2014; Tanko *et al*., 2008). The increase in the plant use, coupled with scarcity of scientific evidence on the safety of this medicinal plant raises concern regarding its toxicity or otherwise on repeated administration.

The medicinal importance of plants has been attributed to the presence of their metabolic products (phytochemical compounds). Ordinarily, these phytochemical compounds are responsible for the biological activity of the plant, and are believed to activate, catalyze or initiate some curative reactions in humans (Uzama *et al*., 2012).The thin layer chromatography profile of the methanol leaf extract of *Securinega virosa* revealed the presence of various compounds with different $R_f$values. Comparing these $R_f$'s with $R_f$'s of known compounds enables tentative identification of the possible compounds to be made.

The phytochemical screening of the methanol leaf extract of *Securinega virosa* revealed the presence of flavonoids, tannins, steroids, saponins, alkaloids,reducing sugars, cardiac glycosides, and anthraquinones. Previous studies have also shown the presence of same phytochemical constituents which could be responsible for the observed pharmacological actions of the plant (Magaji *et al.*,2007a; Yerima *et al*., 2009; Uzama *et al*.,

2012).Alkaloids, flavonoids, saponins and anthraquinones have also been reported to possess toxic potentials (Skibola and Smith, 2000;,Galati*et al*., 2001).

The assessment of acute toxicity of an unknown substance is the first step in toxicological investigation. The most frequently used acute toxicity test involves the determination of median lethal dose ($LD_{50}$). The $LD_{50}$ is a single dose of a material that can kill 50 per cent of animals. The value represents the amount of toxicant per unit weight of the animal, which will kill 50 per cent of a particular population of the animal species exposed to the chemical within a specific time, usually 24-72 hours. The routes of administration and animal species used affect the median lethal dose. The $LD_{50}$ determines the safety margin for any substance or chemical and hence the choice of the doses for pharmacological or biological screening.

Using the Lorke's method (1983), the results of oral and intraperitoneal median $LD_{50}$ value of the methanol leaf extract of *Securinega virosa* in Wistar rats was found to be greater than 5000mg/kg body weight. This suggests that the extract is practically non toxic (Lorke's, 1983). Pascoe *et al.*,(1983)however, classified substances with oral $LD_{50}$ values within the range of 5000 to 15000mg/kg body weight as slightly toxic. The oral $LD_{50}$ value of  5000mg/kg in Wistar rats found in this studywas higher than the value obtained by Magaji *et al.,* (2007b) who found an oral $LD_{50}$ of 1,265mg/kg in mice. The disparate acute oral $LD_{50}$ values observed could be because of possible differences between species in drug metabolism and susceptibility to toxic effects (Salawu *et al.*,2008). Zbinden and Roversi (1981) suggested that variables such as animal species, strain, age, gender, bedding, ambient temperature and time of the day can all affect the $LD_{50}$ value obtained and as such are considerable uncertainties in extrapolating the

$LD_{50}$obtained for species to other species. It was therefore suggested that $LD_{50}$ may not be considered as a biological constant (Zbinden and Reversi, 1981).

The body weight changes serve as a sensitive indication of the general health status of animals. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Grance *et al*, 2008; Nandy and Datta., 2012). In this study, there was a steady increase in weight of both the male and female rats in all the groups over the 28 day period. However, a marginal decrease in weight that was not significant ($p$□0.05), though worth noting, was observed in the group treated with 1000mg/kg body weight when compared with the control group in both male and female animals in the $4^{th}$ week of the study period. The findings in this study is comparable to what was reported in similar other studies (Rhiouani *et al*., 2008; Salawu *et al*., 2009; Nandy and Datta, 2012). The extract at 1000mg/kg body weight may have also been metabolised to a more toxic end product that interferes with appetite and food consumption (Chokshi, 2007).

Organ weight is also an important index of the physiological and pathological status in animals. The relative organ weight is fundamental to establish whether or not the organ was exposed to injury (Jothy *et al*., 2009). There was a statistically significant ($p$□0.05) increase in the weight of the liver and kidneys of both male and female rats treated with 1000mg/kg body weight of extract. This is comparable to a study reported by Salawu and colleagues (Salawu *et al*., 2009)The liver and kidneys being key organs in the metabolism of xenobiotics are vulnerable to damage induced by a wide variety of chemicals (Tennant, 1997; Jothy *et al*., 2009). Thus the increase in relative organ weight of the liver and kidneys in both male and female rats treated with 1000mg/kg body

weight of the extract observed in this study could be attributed to high rate of metabolism resulting from exposure of the organs to the extract.

The haematopoietic system is one of the sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals. Analysis of blood parameters is relevant to risk evaluation as the changes in haematological parameters have a high predictive value for human toxicity, when the data is translated from animal studies (Oslon *et al*., 2000). The haematological parameters (RBC, Haemoglobin, PCV, MCH, MCHC and MCV) showed no significant differences between the control and the treated groups. This finding is in agreement with the studies on *Crossopteryx febrifuga* reported by Salawu *et al.,* (2009), Panunto *et al.,* (2011) and Jaijoy *et al.,* (2011). This suggests that the extract had no effect on circulating blood cells or on their production. The increase in platelets count observed in the study is usually associated with post inflammatory changes or accompanying organ damage (Salawu *et al.,* 2009).

To ascertain whether the defence system of the animals has been compromised, the WBC count as well as the differentials counts was assessed. There wasinsignificant ($p$ 0.05) difference between the control and the treated groups. This is similar to the findings of Nandy and Datta, (2012)in a study on the methanol leaf extract of *Pterospermum acerifolium* in rodents. The white blood cells help to defend the body against infectious disease and foreign materials as part of the immune system (Webb *et al*., 2003).

Liver function tests involve evaluating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP), bilirubin and albumin levels. The

most commonly used indicators of liver damage are the alanine aminotransferase and aspartate aminotransferase (Ramaiah, 2011). These are enzymes normally found in liver cells that leak out of these cells into the blood stream following injury to the liver cells. These liver enzymes become elevated in liver cirrhosis, hepatitis and hepato-biliary obstruction. ALT is localized primarily in the cytosol of hepatocytes and is considered to be a more sensitive marker of hepatocellular damage than AST (Saad *et al*., 2006). It can provide a quantitative assessment of the degree of damaged sustained by the liver (Al-Mamary *et al.*, 2002). In this study, there was a significant ($p$ 0.05) increase in liver markers, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase in both male and female rats treated with 500and1000mg/kg body weight of extract when compared with the control. However, the serum total protein, albumin, direct and indirect bilirubin were not statistically different in the treated groups when compared with their respective controls. This finding is in agreement with Hassan and colleagues (2007), who reported the potential toxicity of anthraquinone glycosides on liver and kidney functions. A study by Galati and co-workers also revealed the toxic potential of flavonoids on isolated rat hepatocytes (Galati *et al*., 2001). The increase in ALT, AST and ALP in this study suggests that 28 days administration of the methanol leaf extract of *Securinega virosa* resulted in hepatocellular injury.

Urea and Creatinine are considered as good prognostic indicators of renal dysfunction and kidney failure for any toxic compound (Ashour *et al*., 2006; Gnamani *et al.*, 2008). As the kidneys become impaired, creatinine level in the blood rises due to damage to the functional nephrons and consequently poor clearance by the kidneys (Aliyu *et al.*, 2006). This will therefore imply a malfunction or failure of the kidneys. In this study, there was

a significant ($p$ 0.05) increase in the serum Urea and Creatinine in both male and female rats in groups treated with 500 and 1000mg/kg body weight of extract compared with control group. This finding is comparable to studies reported by Nandy and Datta, (2012), Salawu *et al*., (2008) and Jaijoy *et al*., (2011). The altered renal indices may be due to the phytochemical content in the methanol leaf extract of *Securinega virosa*. This suggests that the extract could cause adverse effects on the functions of the kidney if given at high doses and for a long period. There may therefore be a need to monitor creatinine levels when the extract is used for treatment of diseases that will require chronic administration. Aliyu and colleagues have however, suggested that for better estimation of renal function, a creatinine clearance test may be required (Aliyu *et al*., 2006).

Histopathological examination is the gold standard for evaluating treatment related pathological changes in tissues and organs (OECD, 2008)

The increase in serum markers of liver and renal function was supported by the histopathological changes observed in the kidneys and liver of the animals treated with 500and 1000mg/kg body weight of extract. The liver of the female rat treated with 250mg/kg body weight of extract however showed some histopathological changes which were not observed in the male rats treated with the same dose of the extract. This might suggest some sex differentiation in the metabolism of the extract that was not pronounced enough to result in concomitant alteration in the serum markers of the animals in that group. Sex-dependentdifferences in xenobiotic metabolism are most pronounced in rats due to differential evolution of cytochrome isoforms in rats (Gregory and Cheryl, 1998). The slower metabolism in female rats produces higher tissue concentrations of xenobiotics that may induce target organ toxicities (Gregory and Cheryl, 1998).

The kidneys of both male and female rats showed dose-dependent toxicities evidenced by focal glomerular atrophy, glomerular damage and vascular congestion in the groups treated with 500 and 1000 mg/kg body weight. These histopathological findings support the raised serum levels of urea and creatinine noted in the groups.Serum creatinine is the most commonly used indicator of renal function because it is excreted unchanged by the kidneys. A rise in serum creatinine level is a late marker, observed only with marked damage to functioning nephrons (Colson and De Broe, 2005).

The observed histopathological findings in the heart of female rats showed myocardial haemorrhages and myocardial thickening in the groups treated with 500 and 1000mg/kg body weight of extract. However, the histopathological sections of the heart in male rats did not show obvious changes that were different from the control across all dose levels. This sex-specific difference may be as a result of the longer half-lives and slower clearance of drugs and chemicals in female rats (Gregory and Cheryl, 1998). Sex differences in pharmacokinetics and pharmacodynamics characterise many drugs and may contribute to individual differences in drug efficacy and toxicity (David and Holloway, 2009). The sexually dimorphic expression of P450s and other liver-expressed genes is regulated by the temporal pattern of plasma growth hormone release by the pituitary gland, which shows significant sex differences. These differences are most pronounced in rats and mice, where plasma growth hormone profiles are highly pulsatile (intermittent) in male animals versus more frequent (nearly continuous) in female animals (Franconi *et al*., 2007).

The observed histopathological lesions across all treatment doses in the lungs and spleen suggests that the extract should be used with extreme caution in individuals with diseases

affecting those organs. The observed histopathological changes (neuronal degeneration and vacuolation) in thecerebellum and cerebrumof rats at doses of 250, 500 and 1000mg/kg body weight of extract after 28 days oral administration also suggests that the extract exerts dose dependent organ toxicities.The solvent system used for the extraction is capable of extracting both polar and non polar constituents and the extracted bioactive hydrophilic components are capable of penetrating the blood brain barrier. This may be responsible for the observed histopathological changes in the brain.The cerebellum and cerebrum cortices are important regions in the brain. The cerebellum coordinates muscular activity and maintains posture and equilibrium (Young *et al*.,2006) while the cerebrum is involved in many of thehigher function such as storage of information,processing of intellectual functions of the brain,characteristic learning and memory (Guyton and Hall,1996).

There were no histopathological changes observed in the reproductive organs of both male and female rats when compared with the control, suggesting that the extract does not have any deleterious effect on the reproductive functions of the animals.

# CHAPTER SIX

## 6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

## 6.1 SUMMARY

The oral and the intraperitoneal median lethal dose ($LD_{50}$) values of the methanol leaf extract of *Securinega virosa*were estimated to be greater than 5000mg/kg bodyweight in rats.The extract had aninsignificant ($p$□0.05) effect on body weight of Wistar rats. The haematological parameters measured were normal except a significant ($p$□0.05) increase in platelets count which was observed at the highest dose.There was a significant ($p$□0.05) increase in liver biomarkers, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase in both male and female rats treated with 500and 1000mg/kg body weight of the extract when compared with the control. There was also a significant ($p$□0.05) increase in the serum urea and creatinine in both male and female rats in groups treated with 500 and 1000 mg/kg body weight of extract compared with control group. Histopathological findings indicated that oral administration of the extract for 28 days resulted in toxicity to the liver (hepatic cell damage characterised by centrilobular necrosis peri-portal hepatocytic vacuolation and scattered inflammatory changes), kidney (glomerular damage and atrophy, tubular epithelial cell necrosis and vascular congestion), and brain (degeneration of nerve fibres, pyknosis and vacuolation) at doses of 500 and 1000mg/kg body weight of the extract while toxicity was observed in the spleen (splenic congestion) and lungs (diffused areas of congestion and inflammation) of both male and female rats across all the doses administered.

## 6.2 CONCLUSION

The present study has enabled the following general conclusionto be made on the toxicity profile of the methanol leaf extract of *Securinega virosa*:

The extract was found to be practically non toxic following acute administration and had aninsignificant effect on body weight changes in rats. Sub-acute (28 days) oral exposure of Wistar rats to the extract did notresult in any effect on circulating red and white blood cells or their production. However, the extract was found to have hepatic toxicity and nephrotoxicity with associated histomorphological changes in the liver, kidneys, spleen, heart, lungs and the brain following oral administration for 28 days. However, the extract had no effect on the histomorphological structure of the reproductive organs (Testes, Ovaries, Uterus) following oral administration for 28 days.

## 6.3 RECOMMENDATIONS

1. The plant extract should be used with caution due to the observed toxicities in the liver, kidney, brain, heart, lungs and spleen

2. A chronic toxicity study should be carried out in same species of animalsusing the leaves, stem bark and roots of the plant.

3. Cardiac enzymes should be assayed given the histomorphological changes observed in the heart.

### 6.4 Contribution To Knowledge

The present study has established that sub-acute oral administration of the methanol leaf extract of *Securinega virosa* produces toxicity to the liver, kidneys, spleen, lungs, heart and the brain, knowledge of which hitherto was not available.

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