HAEMOSTATIC AND HAEMATOLOGICAL STUDIES OF AQUEOUS EXTRACT OF *BIDENS PILOSA* IN WISTAR RATS

BY

ANAELE,ONYEKA

(PG/MSC/11/61021)

BEING A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE OF FACULTY OF HEALTH SCIENCES AND TECHNOLOGY,COLLEGE OF MEDICINE,UNIVERSITY OF NIGERIA ENUGU CAMPUS, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCES (M.Sc) DEGREE IN HAEMATOLOGY.

MAY, 2015.

DECLARATION

I hereby declare that this thesis was written by me and that it is authentic record of my project work. No part of this work has been submitted in any form elsewhere for the award of diploma, certificate or degree.

SIGNATURE:----------

NAME: Anaele, Onyeka

MATRIC NO: PG/MSC/11/61021

DATE: May, 2015

CERTIFICATION

I hereby certify that the research work for this thesis and subsequent preparation of this thesis by ANAELE, ONYEKA (PG/M.Sc/11/61021) were carried out under the supervision of Ven. Prof. E.O. Ukaejiofo, Department of Medical Laboratory Sciences, Faculty of Sciences, University of Nigeria, Enugu Campus.

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VEN.PROF.E.O.UKAEJIOFO DATE

(SUPERVISORõS SIGNATURE)

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PROF. N.F. ONYEMELUKWE DATE

(H.O.DõS SIGNATURE)

DEDICATION

This work is dedicated to my superb wife and children.

ACKNOWLEDGMENT

All adoration and honour are ascribed to Almighty God for His marvelous work in my life. Your plans Oh God are indeed for good. I thank Lord for sustaining me throughout my studies especially for Journey mercies He granted me.

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ABSTRACT

Bidens pilosa L. (Asteraceae) also known as hairy beggar ticks, common names include; *'Ogwunma'; 'Gyeya'*(*Igbo, Birom,* Nigeria) and is one of the medicinal plant species worldwide. Among the *Birom* tribes in Plateau State, Nigeria *Bidens pilosa* is used as vegetable to prepare the local delicacy called *ghote* and also the sap from crushed leaves is used to speed up clotting of blood in fresh wounds. The aim of the study was to evaluate the effect of aqueous extract of *Bidens pilosa leaves* on selected haemostatic and haematological profile in Wistar rats. Phytochemical tests were carried out on *Bidens pilosa* leave extract (BPLE) to detect the presence of metabolites. Lorkeôs method was employed for Acute Toxicity studies to determine the LD50 of the *B.pilosa* extract while in sub-chronic toxicity study, graded doses of BPLE ranging 200-800 mg/kg b.w and distilled water were orally administered daily for 28 days. Sample collection of rats was done prior to administration of extract (Day 0) for base line studies and on days 14 and 28 for evaluation and monitoring of the effect of the aqueous extract of *B.Pilosa* on some selected haemostatic and haematological profile. The haemostatic profile was determined using Prothrombin Time Test(PTT),Activated Partial thromboplatin Time (APTT) and Thrombin Time Test(TT). The haematological profile was determined using Auto Haematology Analyser BC280 Vet. The BPLE was found to contain some metabolites such as flavonoids, cardiac glycosides ,saponins, tannins carbohydrates and alkaloids. No mortality was recorded during the studies in rats. Generally, significant increase at $P<0.05$ in percentage body weight gain was recorded between day 14 and day 28 along each group. The result also showed that the BPLE significantly($P<0.05$) increased Prothrombin time(PT) on day 14 amongst rats treated with 800mg/kg when compared to 400mg/kg group where a significant reduction of PT was noticed.However it was observed that BPLE significantly($P<0.05$) decreased APTT on day 28 among the 800mg/kg group compared to 400mg/kg group. There was a significant($P<0.05$) increase in Thrombin Time(TT) at dose 400mg/kg and 800mg/kg when compared with control and 200mg/kg groups.So this is an indication that *Bidens pilosa* extract has the potential to cause changes on some extrinsic factors and common pathway of clotting factors such as factors I,II and XII when administered at higher dose. This implies that *Bidens pilosa* extract has some anticoagulation properties with respect to PT and TT assay in rats that received 800mg/dl of extract.A significant decrease in APTT values recorded especially on day 14 among all the groups that received *Bidens pilosa* could be attributed to increased activities of some intrinsic factors, suggestive of the procoagulation property of the aqueous extract. However, haemostatic activities of BPL are both dose and dosage dependant. It was observed that there was no significant difference in levels of red blood cell counts in all the groups at $p<0.05$.For haemoglobin (HB) and haematocrit (HCT) concentrations, a significant($P<0.05$) decrease was recorded in rats that received 800mg/kg of BPL when compared with rats that were treated with 400mg/kg of BPLE. Also,MCV and MCH, significantly($P<0.05$) decrease in values among rats that received 800mg/kg of extract when compared to 400mg/kg group.A significant ($P<0.05$) decrease in MCV and MCH values were noted at dose of 200mg/kg when compared with control and 400mg/dl groups respectively. There was a significant ($P<0.05$) increase in WBC among 200mg/kg group compared to control group. However, there were no significant changes in WBC indices amongst other groups. It can be concluded that BPLE on the Pleateau in Nigeria contains bioactive molecules and exerts some haemostatic and haematological changes in Wistar rats.

CHAPTER ONE

1.0 INTRODUCTION

1.1 ORIGIN AND GEOGRAPHIC DISTRIBUTION OF *BIDENS PILOSA*

Bidens pilosa L.(Asteraceae) is a perennial cosmopolitan weed, originating from South America and common in all tropical and subtropical areas of the world climates (Geissberger and Séquin,1991; Alvarez et al., 1999). It is characterized by its hardiness, explosive reproductive potential, and ability to thrive in almost any environment has enabled it to establish throughout the world (fig 1).

Generally introduced unintentionally through agriculture or sometimes intentionally for ornamental purposes, *B. pilosa* is a major crop weed, threat to native fauna, and a physical nuisance. In Africa, *B. pilosa* is recorded as a weed in many countries and it is likely to occur in all countries, including Nigeria. *B. pilosa* is a weed in both field and plantation crops and is recorded as troublesome in about 30 crops in more than 40 countries, including about 20 African countries. It is considered one of the most noxious annual weeds in East Africa (Grombone-Guaratini et al., 2005). It often becomes dominant after the eradication of perennial grasses, and displays allelopathic effects on a number of crops (Lima et al., 2011). Although the plant is an invader and is generally regarded as a nuisance in most countries outside South America, it may possess medicinally important compounds that can be used to treat a variety of ailments.

1.1.1 COMMON NAMES

Bidens pilosa (hairy beggar ticks) (Grubben and Denton, 2004);common names include ó*Mucege'* (*Kikuyu,* Kenya), *'Enyabarashana'* (*Ankole*, Uganda), *'Ogwunma'; 'Gyeya'* (*Igbo, Birom,* Nigeria), ó*Guizhencao'* (China), bur marigold (Europe).

Fig 1 *Bidens pilosa* plant in the premises of National Veterinary Research Institute, Vom, Plateau State, Nigeria.

1.1.2 VARIOUS USES OF *BIDENS PILOSA* PLANT

1.1.3 NUTRITIONAL PURPOSES AND AGRICULTURAL BENEFITS

It is reported as a vegetable or potherb, among others, in Sierra Leone, Liberia, Côte dôIvoire, Benin, Nigeria, Cameroon, Democratic Republic of Congo, Kenya, Uganda, Tanzania, Malawi, Botswana, Zambia, Zimbabwe and Mozambique (Karis and Ryding, 1994).

B. pilosa had strong allelopathic effect which is beneficial in enhancing its capacity in interspecific competition and to promote its invasion (Mao et al., 2010). Aqueous extracts of *B. pilosa* with low concentrations of up to 20 mg/ml had some facilitating effect on bud growth on pasture *Trifolium repens* and *Medicago sativa*, while high concentrations of 100 mg/ml or greater had a considerable inhibitory effect on seed germination and seedling growth. The allelopathic inhibitory effects generally increase with the increase of concentrations (Mao et al., 2010). Cui and He (2009) reported that soil biota and nutrient availability are drivers of the plant invasions.

1.1.4 MEDICINAL PURPOSE

Bidens pilosa L .is one of the dominant families of plants contributing to medicinal species worldwide. Though traditional medicine has been practiced for years in developing countries, especially in Africa, the re-emerging of medicinal plants as health aid in many countries like China and Nigeria has been attributed to historical circumstances and cultural belief in addition to the rising costs and resistance of orthodox drugs in maintenance of personal health and well being (Okeke, 2005). It is a popular folk medicine in Taiwan for all sorts of illnesses, from influenza to hepatitis. In one study with laboratory animals, Taiwanese scientists showed that this herb has significant anti-oedemic and anti-inflammatory activity (Duke, 1997).

1.2. MODES OF PREPARATIONS OF BIDENS *PILOSA* LEAVES FOR USE

Generally, the whole plant is prepared in decoctions or infusions for internal use or crushed into a paste for external use. In Southern and Central America (such as Peru, Mexico and Brazil), *Bidens pilosa* is used for foot-and-mouth disease, angina, diabetes, menstrual disorders, hepatitis, laryngitis, pharyngitis, hemorrhoids, as a gargle for mouth blisters, hepatitis, nervous problems, intestinal worms, for internal and external inflammations, tooth-ache, headaches, sores, lacerations, upset stomach in food poisoning, sore throat and water retention (Taylor, 2005; Duke, 1997). In Uganda, a leaf decoction is used for treating headache; sap from the plant is put in the ear to treat ear infection; a decoction of leaf powder is used to treat kidney problems; and a herbal tea made from the plant decreases flatulence (Tadesse, 1994). It is also used in Uganda by traditional healers in the treatment of opportunistic infections of HIV/AIDS patients mostly oral lesions (Theta, 2005). Its roots, leaves, and seeds are reported to have antibacterial, anti-dysenteric, antiinflammatory, and antimicrobial, antimalarial, diuretic, hepatoprotective, and hypotensive properties (Mvere, 2004; Rojas *et al*., 2006; Wat *et al*., 1980; Dimo *et al*., 2002; Brandao *et al*., 1997; Chiang *et al*., 2004). Although there is some variation in the level of activity of the different species of *Biden*s, probably due to different levels of active constituents, the general properties appear similar (Andrade-Neto *et al*., 2004).

1.3 JUSTIFICATION

Bidens pilosa is used as vegetable to prepare the local delicacy called *ghote* and also the sap from crushed leaves is used to speed up clotting of blood in fresh wounds amongst people of Plateau state, Nigeria. This suggests that *Bidens pilosa* leavesô aqueous extract contains different active phytochemicals responsible for its uses and perhaps has some level of safety. Despite the popular use of this plant in Plateau State Nigeria, there is

dearth of information concerning the toxicity of this local variety. Therefore, this study aim to evaluate the phytochemicals, acute and sub-chronic toxicities and selected haemostatic and haematological effects of aqueous extract of Nigerian *Bidens pilosa*.

Aim

The aim of this study is to evaluate the effect of aqaous extract of *Bidens pilosa* on selected haemostatic and hamatological profile in Wistar rats.

Specific objectives:

1 To ascertain the photochemical and the toxicities of *B.pilosa* species in the locality.

2 To ascertain if the extract has any thrombotic or hemorrhagic tendencies

3 To ascertain if the extract has any effect on some selected haematological profile in Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 AN OVERVIEW OF HAEMOSTASIS

The normal haemostatic system is complex yet exquisitely well regulated. Interrelationships exist between responses of the vasculature, circulating platelets,(primary haemostasis) coagulation proteins,(Secondary Haemostasis) and fibrinolytic mechanism (Tartiary haemostasis). These relationships serve to limit blood loss, preserve tissue perfusion, and stimulate local repair processes. Natural inhibitors of coagulation and fibrinolysis modulate these systems to prevent uncontrolled thrombosis or hemorrhage following pathologic stimuli(Kordich,1992).Vascular endothelial cells play an important role in the maintenance of a thromboresistant luminal interface with circulating cells and proteins. Normal hemostasis also requires the synthetic, metabolic, and repair processes of the vascular endothelium. The initial vascular response to injury produces brief vasoconstriction and exposes subendothelial substances that attract circulating platelets and activate coagulation proteins. Platelets respond by adherence and aggregation at the site of injury, with subsequent release of substances that mitigate blood loss. Platelet adherence to collagen requires von Willebrand's factor, fibrinogen, fibronectin, and specific glycoprotein receptors on platelet surfaces. Platelet-to-platelet interactions (aggregation) recruit additional platelets to the primary hemostatic plug. Aggregation requires fibrinogen, energy, and calcium. Release of ADP, serotonin, and the contents of intracellular granules as well as generation of prostaglandins prepares platelet surfaces for reactions with the coagulation proteins(Heemskerk, 2002) The mechanism of the primary haemostasis is as a result of the platelet transcriptome 6000 mRNA species and represent receptors, ion channels, signalling molecules, kinases, phosphatases, and structural,

metabolic and regulatory proteins. This abundance of regulatory proteins points towards the importance of signal transduction in platelet function. First platelets adhere to collagen, this induces activation signals such as TXA(2) that induces further Ca(2+) increase. Consecutively, fibrinogen binds to the integrin alpha(IIb)beta(3) resulting in aggregation. This self-amplifying process is controlled by signals, from endothelial cells, to restrict the platelet plug to the site of vessel injury (Stassen *et al.,*2004)

Secondary haemostasis (coagulation) consists of an extrinsic and intrinsic pathway (Fig2). Activation of the intrinsic or extrinsic coagulation pathway, or of both, causes formation of fibrin from fibrinogen by means of an elaborate and intricate system that also entraps platelets and activated coagulation proteins. The intrinsic system is activated by the contact of factor XII with a negatively charged surface, most likely collagen. Through a series of reactions with prekallikrein, HMWK, and factors XI, IX, and VIII, the common coagulation pathway is propagated. The extrinsic, or tissue factor, system stimulates both the common pathway and factor IX the intrinsic pathway.

Thrombin is generated via Factor Xa resulting from the extrinsic tenase reaction that is turned off by tissue factor pathway inhibitor. While thrombin generation is maintained via positive feedback mechanisms activating factors V, VIII and XI. Excess thrombin is inhibited by antithrombin or by autodown regulation via activation of protein C. Discovery of this stimulation of the intrinsic pathway by factor VII in the extrinsic pathway has stimulated reassessment of the biologic importance of the extrinsic system. The common pathway includes factors X and V and causes thrombin to convert fibrinogen to fibrin. Since minor injuries are common, platelets and plasma clotting factors constantly produce clots to stop bleeding. If clots remained after the tissue healing, the vascular bed would become obstructed with clots therefore this is regulated by fibrinolysis (tertiary

hemostasis).Tissue-type plasminogen activator synthesised by the endothelium, converts plasminogen to plasmin, the clot lysis enzyme. Plasmin clears the blood vessels by degrading fibrin. Fibrinolysis is controlled by plasminogen activators inhibitor (PAI-1), alpha2-antiplasmin and alpha2-macroglobulin, and thrombin-activatable fibrinolysis inhibitor (TAFI).

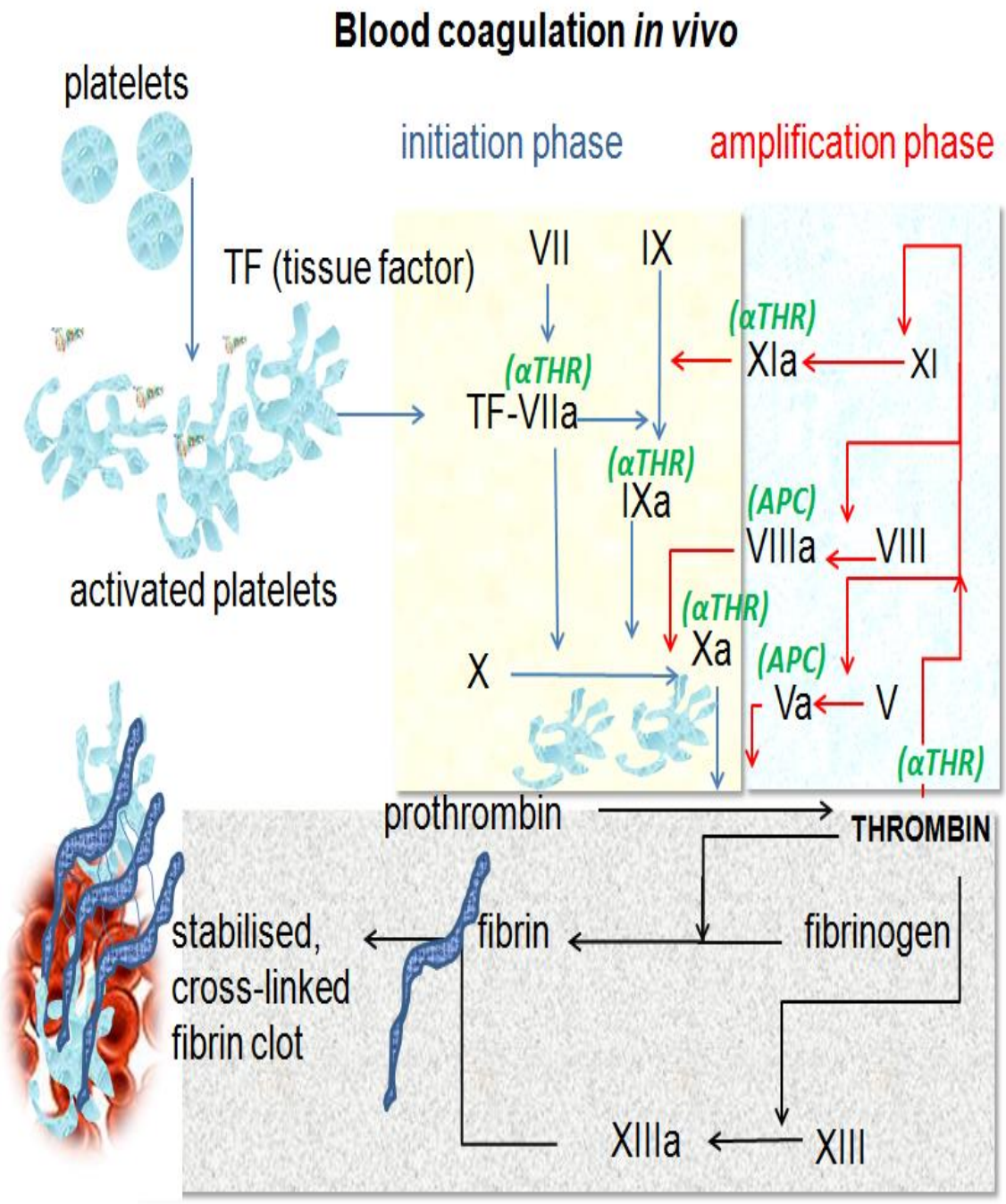

Fig 2 Blood coagulation *in vivo* Adapted from http:/upload.wikmedia.org/wikipedia.

2.2 CONTRIBUTIONS OF RAT PLATELETS AND BLOOD COAGULATION FACTORS TO HAEMOSTASIS

In order to understand some of the haemostatic mechanisms in rats for the interpretation of toxicological data, basic haemostatic parameters with a special emphasis on platelet functions were first measured in vitro. According to research carried out by Takahashi (2000),the results of reactions of rat platelets to many aggregating agents suggest that only ADP may be a consistently significant aggregator. The search for physiologic aggregators revealed ADP to be available from erythrocytes. Adhesion reaction also required ADP. Collagen was not considered to be essential for either reaction. Aggregation and adhesion were probably both reversible in flowing blood, while irreversible thrombi were formed in blood at rest ex vivo.

Blood coagulation parameters determined revealed that the intrinsic pathway may be more important than the extrinsic one. The rate of intrinsic coagulation reaction was rapid, and plasma coagulation appeared to be of primary importance while the influence of platelet aggregation was minor. A simple model of rat haemostatic mechanism is proposed based on these results. Additionally, to define the relative contribution of platelets versus other cellular and plasma coagulation in vivo, rats were administered antiplatelet drugs (ticlopidine, suprofen and clopidogrel) and an anticoagulant (warfarin) intraperitoneally. Bleeding times (BTs) were significantly increased in all treated groups. ADP-induced platelet aggregations were significantly depressed by the administration of the three antiplatelet drugs, while kaolin-activated partial thromboplastin time and prothrombin time were greatly increased in the warfarin-treated rats. The increase in BT may be due to the inhibition of platelet activity or blood coagulation defect in rats given antiplatelet drugs or warfarin, respectively. These results suggest that platelets play a key role in

haemostasis in the rat. Two possible explanations of the disparity between in vitro and in vivo results may be that functional tests used here are not adequate to cover the properties of rat platelets or that mechanisms leading to the formation of platelet thrombi in rats are ADP-dependent adhesion and ADP-induced aggregation

2.3 REFERENCE VALUES OF HAEMOSTATIC PARAMETERS IN MALE WISTER RATS.

Information in various literatures regarding studies on blood clotting parameters performed in laboratory animals have been centered on effects various of medicinal plants extracts on haemostatic profile of rats (Tanko *et al*, 2012; Alhamanami *et al*; 2006;) There is little information on the standardization of rat blood clotting tests with reagents used for humans besides the work of Garcia-Manzano *et al*(2001).

The Table 1 shows the coagulation tests of normal Male Wister rat as reported by Garcia-Manzano *et al;*(2001).

Test	Mean of Test(±SEM)	Minimal-Maximal Values	Coefficient of variation	No of Test(Rats)
Blood Clotting Time(S)	125±3.86	113-136	6.90	10
Bleeding Time(S)	88±4.0	60-110	17.6	14
Prothrombin Time(S)	26.7±0.4	24.5-30.9	6.80	23
Activated Partial Thromboplastin Time(S)	16.5±0.4	13.0-19.2	11.2	23
Thrombin Time(S)	31.1±0.5	28.6-35.8	6.87	21
Fibrinogen(mg/dl)	200.9±5.1	160-255	11.8	22

2.4 FACTORS INFLUENCING CHANGES IN HAEMOSTATIC PARAMETERS IN RATS

2.41 PHYSICAL ACTIVITY

Studies have shown that there is a relationship between physical activities and levels of some haemostatic parameters (Hilberg *et al*.,2003;2005).Based on these findings, a number of investigators have suggested that there is functional relationship between the pineal gland, via hormone meltonin and the coagulation system (Tunali *et al*., 2005).In that regards, report has shown that the chronobiological patterns should be considered when analyzing activity levels of coagulation factors (Pinotti *et al*., 2005).The intensity of

acute exercise is a critical factor affecting blood platelets function(Wang *et al*.,1997).In addition reports has shown an increased platelets counts of 18 to 80% immediately after treatmill or bicycle exercise(EL-sayed *et al*., 2005).Interestingly, there is possibility that thrombin is involved in the platelets activation induced by strenuous exercise. Also exercise enhances blood coagulation and fibrinolysis, as shown by elevated plasma levels of prothrombin fragment and tissue plasminogen activation (Nailin *et al*., 2007).In epiphysectomized (EP) rats, there is increase in thrombin time. Interestingly the clotting time increased after a long time exercise but decreased after short-time exercise in epiphysectomized EP) rats (Rostami *et al*., 2011)

2.4.2 INFLUENCE OF SOME MEDICINAL PLANTS EXTRACTS ON HAEMOSTATIC PARAMETERS

2.4.2.1 GARLIC

Garlic is a plant member of the lily family closely related to onion. The important ingredient of garlic is alliin. The allinase, other ingredient of garlic, turn alliin to allicin which is responsible for garlic odor and plays a major role in lowering cholesterol (Washafsky *et al*.,1993;Silagy *et al*.,1994). It was shown, in vitro studies, that garlic also contains s-allyl cyste in (SAC) and di-allyl-disulfide (DADS) which are potent inhibitors of cholesterol synthesis (Yu *et al*.,2001) The antihypertensive effect of garlic is attributed to its prostaglandin like effect that is leading to decrease in the peripheral vascular resistance. The gamma-glutamyl cysteines, one of the active ingredients of garlic, may decrease blood pressure by its ability to inhibit angiotensin converting enzyme (Sandle et al.,1992) as well as elicits nitric oxide action and hence having pulmonary artery relaxant effect (Fallon *et al.,* 1998;Kim-Park *et al.,* 2000).

Garlic has shown to reduce the fibrinogen level in hyperlipidemic rats and this has been attributed to its fibrinolytic activity. So the fibrinolytic activity of garlic, therefore, is a favourable antithrombotic effect. Thus leading to the prolongation of APTT and PT in hyperlipidemic rats after 4 weeks of treatment with garlic.(Alhamanami *et al.*,2006).In addition garlic was shown to lower the platelets counts and inhibits platelet aggregation. This could be attributed to ability of garlic to inhibit adenosine diphosphate (ADP), collagen, arachindonate ,epinephrine, calcium ionophore as well as inhibits the formation of thromboxane, phospholipase and lipoxygenase formed in the platelets (Apitz and Ledezma, 1986).

2.4.2.2 MUSHROOM (*GANODERMA lUCIDUM*)

The haemostatic effect of aqueous extract of mushroom (*Ganoderma lucidium*) in rats has been investigated. According to the Tanko et al(2012),aqueous extract of the mushroom significantly decreased bleeding time at different doses of 200mg/b.w to 800mg/b.w after 3rd day administration. Conversely, there was no significant decrease of bleeding time after 10th day administration. Similarly, the findings of Bamidele *et al*.,(2010),revealed that methanol extract of *Ageratum conyzoides* significantly decreased bleeding and clotting time in albino wister rats. The preliminary photochemical screening of *Ganoderma lucidium carried* out by Mohammed *et al*.,(2009) showed the presence of Tannins, Flavonoides, and alkaloids. According to Okoli *et al*.,(2007),tannins a bioactive ingredients of many medicinal plants has been implicated in haemostatic activity through their involvement in the arrest of bleeding from damaged or injured vessels by precipitating proteins to form vascular plug.

In a related study on the efficacy of topical application of medicinal plants for treatment of bleeding a drug known as Ankafered Blood stopper$^{(R)}$ (ABS) has been

evaluated. This ankafered comprises a standardized mixture of plants *Thymus vulgaris, Vitis vinifera, Alpinia officinarum* and *urtica dioica* known as ankafered Blood stopper(R) (ABS) .ABS is medicinal product that has been approved in the management of external haemorrhage and dental surgery bleedings in Turkey.The result of the findings of evaluation of the effect of haemostatic parameters in rats pretreated with warfarin revealed that topical ABS administration to amputed leg shortened the duration of bleeding markedly in both untreated and warfrin-treated rats by 31.9% and 43.5% respectively(Cipil *et al.*, 2009). Similarly, the finding on the evaluation of the changes on haemostatic parameters induced by valdecoxib, a cyclooxygenase-2 selective inhibitors indicated that valdecoxib induced significant activated partial thromboplatin time reduction after 2 weeks and prothrombin time reduction after 3 weeks (Froza *et al.*,2006).

In addition, the influence of captoril on some haemostatic parameters in rats with ongoing process of venous thrombosis, strongly suggest that rennin-angiotensin system plays an important role in regulation of haemostasis and fibrinolysis especially in exerting antithrombotic effect in venous thrombosis in rats. It was further demonstrated that this effect is not as a result of changes in platelet count, fibrinogen level alteration in blood coagulation pathways and fibrinolytic activity of plasma(Pawlak *et al*., 1996).

2.4.3 AGE AND DIET

The roles of age and diet on some changes in haemostatic parameters have been evaluated. Age-related changes in coagulation, fibrinolysis and platelets aggregation in male rats investigated showed that rats aged 6 months or more have significant hyperglycaemia and hyperlipidemia and as result of these biochemical changes, there were

increase in factors II,V,VII,VIII,IX,X and XII activites., a significant decrease in antithrombin III activity in rats (Nobukata *et al.*,2000)

The role of dietary fatty acids on postprandial activation of haemoatatic factors showed that there a relationship between elatvated plasminogen activator inhibitor-1 and elevated fasting plasma triacylglycerol concentration, obesity, and insulin resistance syndrome. Fat- modified diet results in increased fibrinolytic activity (Asplund-Carson *et al.*, 1993).Recent finding has indicated that in postprandial state, large triacylglycerol-rich particles can support the assembly and function of coagulation complexes and seem to play a role in the activation of factor VII,PAI-1 and platelets, and thus may explain increased in cardiovascular disease(CVD) risk associated with increased postprandial triglyceridemia (Duttaroy, 2005).

2. 5 POTENTIALS OF *BIDENS PILOSA*

Herbal drugs from natural sources like plants are in use since the traditional system of medicine; they represent a precious reservoir of innovative bioactive molecules. Among them *Bidens pilosa* Leaf is one of the important plant found in all tropical and subtropical region of the world. It is the herbaceous flowering plant, having white 'petals' around a intense bunch of orange florets. Although considered an invader in many countries, the potentials benefits may outweigh the risks that the weed poses to the environment(Arthur *et al* .,2012).Thus, it has been reported to possess effective pharmacological properties like antibacterial activity(Ashafa and Afolayan,2009) anti-inflammatory and antiallergic activity(Lermen *et al.,* 2012;Chin,1995), antimalarial Activity(Andrade-Neto *et al.*,2001; Brandao *et al.*,1997) T helper cell modulator, immunosuppressive antihyperglycemic, anti-

hypertensive, antiulcerogenic, hepatoprotective (Chin,1996), anti-leukemic, anticancer (Nakama *et al.*,2011) antipyretic, anti-virus, anti-angiogenic, antirheumatic, antibiotic (Khemral *et al.*, 2010). It has shown to have pontentials as a labour facilitator during child birth (Longo *et al.*, 2008). *B.pilosa* leaf extract showed antiulcer activity against indomethacin-induced gastric lesions. It also exerts a cytoprotective effect in addition to its gastric antisecretory activity that could be due, partly to the presence of flavonoids, of which quercetin, was identified by high performance liquid chromatography (HPLC)(Alvarez *et al.*,1999).

2.6 PHYTOCHEMISTRY OF *BIDENS PILOSA*

The Bidens pilosa L. has various chemical constituents like polyacetylenes (Sundararajan *et al.,* 2006). Polyacetylenic glycosides, aurons, auron glycosides, p-coumeric acid derivatives, caffeoylquinic acid derivatives, pheophytins, diterpenes, tannins, phytosterols, ascorbic acid, carotene, essential oils, saponins, steroids and flavonoids and many others were recognized in this plant(Ezeonwumelu *et al.*,2011).It has been revealed that the chemical composition of the extract in 1mg/100g b.w caused 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities which were comparable to those of ascorbic acid. This shows that the extract has the proton-donating ability and could serve as free radical inhibitor or scavenger, acting possibly as primary antioxidant(Adedapo *et al.*,2011)

2.7 TOXICOLOGICAL STUDIES OF *BIDENS PILOSA* AQUEOUS LEAVES EXTRACT

Research carried out by Cardenas *et al.,*(2006), revealed that the infusion of *Bidens pilosa* does not produce acute oral toxicity at a limit dose of 200mg/kg and does not neither produce toxicity after the application of repeated doses of 1000mg/kg during

28days,being produced some beneficial changes on haematological and biochemical variables in the sentinel group. Conversely,the effects of intraperitoneal administration of chromatographic fraction isolated from the aqueous leaf extract of *Bidens pilosa* investigated in mice ,isolated rat heart and rabbit ECG indicated LD50 of 429.14±28.11mg/kg b.w. The result of this study indicated that *Bidens pilosa* was relatively toxic but could be used with caution (Kouakou *et al.*,2013)

2.8 BIOCHEMICAL AND HISTOLOGICAL ACTIVITIES OF AQUEOUS *BIDENS PILOSA* LEAVES EXTRACT

Selected serum enzymes levels of rats after 28 days of treatment with aqueous extract of *Bidens pilosa* leaves (BPLE) revealed significant increase in the serum levels of Alanin Aminnotransferase (ALT), Aspartate Aminotransferase (AST), Gamma glutamyl transpeptidase (GGT) and creatine kinase (CK-MB) enzymes at dose of 800mg/kg b.w when compared with control (Ezeonwumelu *et al.,* 2011).Significant increase in the levels of the enzymes was dose dependant indicating that graded doses of BPL extract could have caused damage in the liver and heart tissues thereby releasing enzymes from these organs to increase the serum levels of these enzymes. So the increase in serum levels of the these selected enzymes is an indication that short term use of the BPL extract could have damage to liver and heart organs. However, it has been reported that the PBL extract is hepatoprotective (Suzigan *et al*., 2009; Li-Ping *et al.*, 2008),its long term administration at high dose may constitute a challenge to the body though, may resolve post treatment (Ilodigwe *et al.*,2010).However there is dearth of information on the haemostatic and haematological profile of aqueous extract of *Bidens pilosa* in rats .So this work is designed to evaluate the effect of aqueous extract of *Bidens pilosa* leaves using haemostatic and haematological parameters as indices.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.11 Some special equipment and materials

Auto Haematology Analyser BC2800Vet, stopwatch, water bath, 12×75mm glass test tube, automatic pipette, manual weighing balance, plastic tube, centrifuge, centrifuge tubes, test tubes racks.

3.12 Plant Material *Bidens pilosa* was collected from the premises of National Veterinary Research Institute, Vom, Plateau State.

3.13 Chemical and reagents

Reagent-calcium Rabbit Brain Thromboplastin, Activated Partial Time Test Particulate Activator(APPT-P) Calcium chloride 0.02mol/l, Bovine thrombin.3.8% trisodium citrate.Ethylene diamine tetraacetic acid(EDTA)

3.2 METHODOLOGY

3.2.1 COLLECTION AND IDENTIFICATION OF *BIDENS PILOSA*

Fresh leaves of *Bidens pilosa* were collected in April 2013 early in the morning during the rainy season around premises of National Veterinary Research Institute,Vom,Plateau State,Nigeria.The *Bidens pilosa* plant was identified by Mr Agyeno O.E. a botanist from Department of Pant science and Technology,Faculty of Natuaral Sciences,University of Jos,Plateau State,Nigeria.The voucher specimen with identification number FHJ022 was confirmed by Mr Jeffery J.Azila of Herberium Unit,Federal College of Forestry,Jos,Plateau State.

3.2.2 PREPARATION OF *BIDENS PILOSA* LEAVES EXTRACT

A voucher specimen of the plant with identification number FHJ022 was deposited in Herbarium of the Biochemistry Department of Federal College of Animal Health and Production Technology,National Veterinary Research Institute,Vom,Plateau State,Nigeria for preparation of *Bidens pilosa* Leaves Extract.

3.2.2.1 DECOCTION METHOD OF EXTRACTION (As described by Ezeonwumelu, *et al.* ,2011)

The leaves of *Bidens pilosa* plant collected was plunged out from the plants, dried under shade and ground into fine powder and decoction method of extraction was used; 400g of the powder was weighed into an empty clean beaker and 1litre of distilled water added and boiled for 4 hours. It was allowed to cool at room temperature and sieved using a clean cotton cloth and subsequently filtered through Whatmann No. 1 filter paper plugged in a funnel. The filtrate was poured into clean dry beakers, held in the hot air oven and evaporated to dryness at 80^{o}C until a constant weight is achieved.

3.2.3 PREMINARY PHTOCHEMICAL SCREENING

Various qualitative phytochemical screening for resins, alkaloids, flavonoids, anthroquinin, glycosides, steroids, terpens, cardiac glycosides, saponins and carbohydrates were analyzed in the aqueous *Biden pilosa* (BP)extract using standard methods of Trease and Evans,(2002)

3.2.4 LABORATORY ANIMAL ACQUISITION AND MAINTENANCE

Wistar rats bred in the Animal Farm of Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State was used for the study. Thirty six health

male and female Wistar rats weighing between 81grams and 208grams obtained were housed separately according sex and weight for 2 weeks to allow for acclimatization. The Wistar rats with relatively similar weights were kept in the same cage and there were housed in a roofed blocked building at room temperature with adequate ventilation, under a naturally illuminated environment with 12 hours of light and 12 hours of darkness. They were fed with standard diet (Pellet feed® Daqwom farms, National Veterinary Research Institute, Vom Plateau State,Nigeria).All the animals had access to clean drinking water and feed *ad libitum* and physically examined and weighed during the period of acclimatization. All the animals with physical anomaly, evidence of infection were excluded from the study. The animal experiments was conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH,2011) and permission for approval for laboratory Space for the animal experiments was sort and obtained from Nigerian Institute for Trypanomiasis Research,Vom Outstation.(see appendix 1)

3.3 ACUTE TOXICITY STUDY

This test was conducted in two phases according to the Lorkeôs (1983) method for the evaluation of the safety of herbal medicines.

In phase I,

Nine male and female young adult Wistar rats were randomly divided into three groups of three animals each. The animals were deprived of food for 16-18hours prior to administration of the extract. The rats were weighed and grouped in three groups as follows:

Treatment groups (group A for low dose of 10mg/kg),

Group B for medium dose of 100mg/kg and

Group C for high dose of 1000 mg/kg and observed for signs of toxicity immediately and up to 72 hours after administration.

In Phase II,

Three male young adult Wistar rats were divided into three groups of one animal each. The animals were deprived of food for 16-18h prior to administration of the extract. They were also weighed and grouped into three groups as follows: Treatment groups

Group A for low dose of 2,000 mg/kg),

Group B for medium dose of 3,000 mg/kg and

Group C for high dose of 5,000 mg/kg

3.4 SUB-CHRONIC TOXICITY TEST:

As Described by Organization for Economic Cooperation and Development (OECD) Test Guidelines (TG) which describe short-term repeat-dose toxicity testing: Repeated Dose 28-day Oral Toxicity Study in Rodents (TG407) was used for the study (OECD, 2006).Twenty four healthy male and female Wistar rats were weighed and grouped according to weights into four groups (n =6) as follows: The treatment groups which include eighteen rats of three groups;

Group A for low dose (200 mg/kg),

Group B for medium dose (400 mg/kg) and

Group C for high dose (800 mg/kg);

Control group comprised six rats to which clean water was administered. They were given food after administration of the extract by gavage. Body weights of the animals were taken weekly and administration of the extracts were done daily for 28 days The rats were observed daily to detect differences in appearance, discoloured fur, diarrhoea, bloody

stool and constipation, loss of appetite and thirst and lack of interest in the environment. After 28 days, all surviving animals, blood samples were collected for haemostatic and haematological studies.

3.5 SAMPLE COLLECTION:

This was done as follows; pretreatment, during and after treatment

Prior to administration of Extract (Day 0) for base line studies and Days 14, and 28 for evaluation and monitoring of the effect of the aqueous extract of *B.pilosa.* Out of 4mls blood samples collected from each animal from the ophthalmic venous plexus located in the orbital sinus of the rats using a micro-capillary pipette (Stone, 1954), as modified by Riley (1960),2mls were dispensed immediately into plastic tube containing 3.8% sodium citrate to give the anticoagulant-blood ratio of 1:9.The blood was mixed gently and centrifuged and the plasma was used for determination of haemostatic parameters and 2mls was dispensed into disposable tube containing 0.04ml of EDTA for determination of haematological parameters.

3.6 STUDY LOCATION

All samples were analyzed in the Department of Haematology, Federal College of Veterinary and Medical Laboratory Technology,Vom,Plateau state.

3.7 HAEMOSTATIC ANALYSIS

The blood collected into citrated tubes was mixed gently and centrifuged at 2500g for 15mins at room temperature the plasma separated and analyzed for some selected haemostatic profiles; Prothrombin time(PT),Activated Partial Thromboplastin Time(

APTT) and Thrombin Time (TT) according to manufacturing instruction and as described by Lewis *et al*(2006) as follows;

3.8 TECHNIQUES ADOPTED IN RUNNING THE SAMPLES

3.8.1 ONE STAGE PROTHROMBIN TIME TEST (As described by Lewis *et al*., 2006)(Reagent- calcium Rabbit Thromboplastin by Diagen Ltd,UK.)

Principle

Thromboplastin activates the coagulation factors of the extrinsic system in plasma in the presence of calcium ions. The clotting time depends on the concentration of factors II, V, VII, and X.A prolonged clotting time indicates deficiency of one or several of these factors .

Materials and Reagents:

1. Reagent-calcium Rabbit Brain Thromboplastin by Diagen Ltd
2. Platelet poor plasma (samples and control

Procedure:

1. 0.1ml of plasma was pipetted into a clean 12×75mm glass test tube placed in a waterbath.
2. 0.2ml of calcium rabbit brain thromboplastine pre-warmed at 37^{o}C for 5 minutes was added and simultaneously a stop was started.
3. It was observed, tilted at intervals for clot formation and the stopwatch was stopped at the appearance of the first fibrin web.
4. The procedure was repeated for each sample and the average of the two results was recorded as the PT
5. The controls were analyzed as tests.

   Normal value = 10 ï 15 seconds

3.8.2 ACTIVATED PARTIAL THROMBOPLASTIN TIME (As described by Lewis *et al.*,2006)

Principle

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastine and so indicates the overall efficiency of the intrinsic pathway. The reagent used contains phospholipids and magnesium aluminum silica as the activator for optimum sensitive factor deficiencies and to heparin. So the reagent activates the coagulation factors of the intrinsic system in presence of calcium ion. The clotting time depends on the activity of factors VIII, IX, XI, and XII as well as I, II, V and X.

Material and Reagents:

1. Activated Partial Time Test Particulate Activator(APPT-P) by PZ Cormay, Poland.
2. Calcium chloride 0.02mol/l
3. Platelet poor plasma (patient samples & control).

Procedure

1. 0.1ml of plasma was pipetted into a clean 12×75mm glass test tube placed in a water bath.
2. 0.1ml of APPT reagent was added to the test plasma and was mixed incubated for 5mins (Activation Time).
3. Then after 5min activation time,0.1ml of prewarmed 0.025M Calcium chloride was forcibly added.

4 Stopwatch was started simultaneously and the mixture was observed for clot formation

5. The stopwatch was stopped and the time noted at the appearance of the first fibrin web.

7. The procedure was repeated for each sample to get a duplicate result and the average of the two was recorded for APTT

8. The controls were analyzed as tests
Normal value = 26 ï 40 seconds

3.8.3 THROMBIN TIME.(As described by Lewis *et al*.,2006)

Principle

Thrombin is the enzyme and penultimate protein in the clotting sequence, it acts upon the soluble fibrinogen converting it to insoluble fibrin.Diagen Bovine thrombine can be used in the laboratory evaluation of fibrinogen disorders, including hypofibrinogenemia and dysfibrinogenemia.

Material and Reagents:

1. Bovine thrombin prepared by Diagen Ltd.Uk.
2. Platelet poor plasma (patient samples & control).

Procedure

1. 0.2ml of plasma was pipetted into a clean 12×75mm glass test tube placed in a waterbath.
2. Then 0.1ml of Bovine Thrombin was added and

3 Stopwatch was started simultaneously and the mixture was observed for clot formation

4 The stopwatch was stopped and the time noted at the appearance of the first fibrin web.

5 The procedure was repeated for each sample to get a duplicate result and the average of the two was recorded for TT

6. The controls were analyzed as tests

(Normal Values 15-19sec)

3.9 HAEMATOLOGICAL ANALYSIS

The blood collected into EDTA tubes was mixed properly and some selected haematological profiles were analyzed using Auto Haematology Analyser BC2800Vet, Shenzhen Mindray, China ;

Packed cell Volume(PCV), Haemoglobin estimation(HGB) Platelets(PLT), Total white cell count(WBC),Total red count(RBC),Mean Cell Volume(MCV),Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration(MCHC).Mean Platelet Volume(MPV), Platetocrit(PCT). Platelet Distribution Width (PDW),Granulocytes, Monocytes and Lymphocytes.

METHOD OF DATA ANALYSIS

The imputed data were exported into IBM Statistical Package for the Social Sciences (IBM SPSS) version 20.0 software for analysis. Analysis carried out included means and standard deviations of the various parameters. Mean difference of the parameters were tested using Analysis of Variance (ANOVA) and Studentôs t-test where appropriate. Statistical significance was set at $p<0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 PHTOCHEMICAL ANALYSIS.

Table 4.1 shows that the phytochemical screening of *Bidens pilosa* leaf(BPL) extract tested positive for saponins, flavonoids, cardiac glycosides, carbohydrates, tannins, steroids, anthraquinones.

4.2 ACUTE TOXICITY EVALUATION

No incidence of animal death recorded in both phases 1 and 11 indicating no toxicity. Also there were no differences in appearance, no discoloured fur, diarrhea, bloody stool, constipation, anorexia. It was observed that during phase 11 of the experiment the animals showed lack of interest in environment for 5 minutes.

4.3 SUB-CHRONIC TOXICITY STUDY FOR 28 DAYS

The study revealed that there were no differences in appearance, no discoloured fur, no diarrhoea ,no bloody stool, no constipation and lack of appetite, thirst and interest in the environment among the rats. However, some animals showed lack of interest in taking the extract.

Table 4.2 shows the mean weight values of rats over 28 days of treatment with aqueous *Bidens pilosa* leaf(BPL*)* extract. During sub-chronic study, no motality was recorded indicating the safety profile of BPL in experimental animals and by extension humans. Increase in body weights was observed in control and in rats treated with 800mg/kg of aqueous BPL. However, reduction in body weight were recorded on day 14 among 200mg/kg and 400mg/kg groups of rats. But increased in body weight were recorded along each group of rats from day 14 to day 28.

Table 4.3 indicates percentage gain in body weights of experimental rats. Body weight gain was observed in control and 800mg/kg group of rats on day 14.In contrast,

there was a reduction in percentage body weight in 200mg/kg and 400mg/kg of rats on the same day 14.But generally, significant increase at P<0.05 in percentage body weight gain were recorded between day 14 and day 28 along each group. However, there was a significant body weight lose at P<0.05 on day 14 between the control group(14.07g),200mg/kg group(-5.83g) and 400mg/kg group(-6.00g).But a significant increase in percentage body weight at P<0.05 was observed between 400mg/kg group(-6.00g) and 800mg/kg group(2.65g) on the same day 14.There was a significant lose in percentage body weight on day 28 at P<0.05 between control group(24.66g) and 400mg/kg group(-3.08g).

Table 4.4 shows the mean and standard deviation of haemostatic indices of Wistar rats administered with graded doses of aqueous extract of *Bidens pilosa* and control. There was significant(P<0.05) increase in Prothrombin Time(PT) on day 14 among 800mg/kg group(17.17sec) when compared with the 400mg/kg group(10.17 sec). Similarly, a significant increase (P<0.05) in Activated Partial Thromboplastin Time(APTT) was noticed on day 0 at dose 800mg/kg(32.52 sec) compared to rats that received 400mg/kg b.w.(18.79 sec) .Interestingly, there was significant decrease (P<0.05) in APTT on day 28 among 800mg/kg group(14.60sec) as against 400mg/kg group (28.94sec).However, thrombin time(TT) revealed a significant(P<0.05) increase on day 28 among 400mg/kg group(19.72sec) when compared with the control group(15.35 sec) and 200mg/kg group(15.09sec).

Table 4.5 shows the mean and standard deviation of Haemostatic indices in relation to duration in Wistar rats within treatment group. There was significant (P<0.05) increase in PT among the 200mg/kg group on day 14(15.4±4.2sec) and day 28(16.02±.4 sec) when compared with day 0(10.3±2.4sec).Similarly, PT values increased

significantly(P<0.05) among rats that received 800mg/kg of BPL extract on day 14(17.2+2.7) compared to day 0(14.4±5.9).Conversely,APPT values decreased significantly(P<0.05) among the 800mg/kg group on day 14(22.0±15.2 sec) and 28(18.2±6.2sec) when compared with day 0(30.7±15.3).Similarly, a significant decrease(P<0.05) was observed on day 14(12.2±6.2 and day 28(14.6±4.4) when compared to day 0(32.5±15).Interestingly,there was no significant diference(P>0.05) in Thrombin Time value among the groups with respect to duration of treatment.

Table 4.6 indicates the Mean(±STD) of thrombocyte Indices of Wistar rats administered with graded doses of aqueous extract of *Bidens Pilosa* leaves(BPL) and Control.The result revealed that all parameters increased with increased in dose from control to 400mg/kg group but a slight decrease in MPV and PDW among the 800mg/kg group was observed. However, there was no significant differences at P<0.05 in all treatments groups.

Table 4.7 reveals the mean (±std) of thrombocyte indices in relation to duration in rats within treatment groups. Unlike the table 4.6 that showed no significant differences between each treatment or groups, there were significant differences recorded in almost all treatments with respect to duration. The platelets counts showed a significant (P<0.05) increase among the control groups on days 14 and 28 when compared with day 0. Also among the 400mg/kg and 800mg/kg groups the same pattern of increase in platelets counts were observed at P<0.05 as duration of treatments progressed from Day 0 to Day 28. However, among the 200mg/kg group the result showed a significant(P<0.05) increase in platelets count on day 14 but a decreased platelets count on day 28. Mean platelets volume (MPV) increased significantly at P>0.05 among the control group on day 14 as against day 0 and day 28. Similarly, significant (P<0.05) increase in MPV was observed

among the 400mg/kg group on day 14 when compared with day 0 and 28. However, those of 200mg/kg and 800mg/kg groups, no significant increase in MPV was recorded during the duration of treatments.

Amazingly, the Platelets distribution width(PDW) increased significantly at $P<0.05$ from day 0 to day 28 among control ,200mg/kg and 800mg/kg groups while among 400mg/kg groups all the days were significantly different from each other at $P<0.05$.

The Platetocrit (PCT) values significantly($P<0.05$) increased among the control and 800mg/kg groups on days 14 and 28 when compared with day 0,while among the 200mg/kg and 400mg/kg groups, PCT significantly($P<0.05$) increased only on day 14 when compared with days 0 and 28.

Table 4.9 indicates the effect of BPL on leucocytes indices in relation to duration within each treatment. White blood cell count (WBC) showed no significant difference in all the groups with respect to duration. However, there was a significant ($P<0.05$) decrease in the percentage of lymphocytes among the control groups on day 14 when compared with day 0 and day 28. For among the 400mg/kg group a significant ($P<0.05$) decrease in percentage of lymphocytes was observed only on day 28 when compared with days 0 and 14. While among the 800mg/kg group a significant ($P<0.05$) decrease in lymphocytes percentage was noticed on days 14 and 28 day when compared with day 0

There was significant ($P<0.05$) increase in percentage of monocytes, on days 14 and 28 when compared with day 0 in all the groups.

Taking a look at effect of BPL on granulocytes, it was noted that among the control group, day 14 was significantly different from Day 0 and Day 28 while among 400mg/kg group, the same day 14 was significantly different from day 28 only

Table 4.10 shows the mean(±std)of erythrocyte Indices of Wistar rats administered with graded doses of aqueous extract of *Bidens Pilosa* leaves(BPL)and control. It was observed that there was no significant difference in levels of red blood cell counts in all the groups at $p<0.05$. For hemoglobin and haemotocrit concentrations, a significant ($P<0.05$) decrease was noted in rats that received 800mg/kg of BPL when compared to rats that were treated with 400mg/kg of BPL.

Considering the Mean Cell Volume (MCV) and Mean Cell Hemoglobin concentration, it is interesting to note that , there were significant($P<0.05$) decrease in values of these parameters among rats that received 800mg/kg of extract when compared to 400mg/kg group. It was observed that, among the 200mg/kg group ,MCV values decreased significantly when compared to control group, while the Mean cell Haemoglobin concentration (MCHC) also decreased significantly ($P<0.05$) when compared to 400mg/kg group .However, for mean cell haemoglobin concentration (MCHC) and red cell distribution width(RDW) no noticeable significant difference among the groups was recorded.

Table 4.11 indicates the effect of BPL on erythrocyte indices with respect to duration in each treatment group.

Unlike the table 4.10 where it was observed that there were no significant different in red blood cell counts ,among the different treatment groups, in table 4.11, there was noticeable changes in red cell counts among 200mg/kg, 400mg/kg and 800mg/kg groups, where it was observed that on Day 14 red cell counts increased significantly($P<0.05$) when compared with day 28. The table 4.11 also revealed a significant increase in Hemoglobin concentration($P<0.05$) on day 14 amongst 200mg/kg,400mg/kg and 800mg/kg groups compared to days 0 and 28. There was a significant increase in haematocrit

(HCT)($P<0.05$) on day 14 among all the treatment groups compared to day 0 and 28.There was also significant increase in MCV($P<0.05$) on day 28 among control group compared to days 0 and 14.In contrast, among the rats treated with 800mg/kg , there was a significant decrease in MCV ($P<0.05$) on day 0 compared to day 14 and day 28.Mean cell haemoglobin (MCH) values increased significantly($P<0.05$) on day 28 when compared to day 0 while among the 200mg/kg group the significant($P<0.05$) increase in MCH was recorded on day 14.Also among the rats treated with 400mg/kg of extract, a significant increase in MCH was recorded on day 14, however, there were significant different among all the days. Rats that received 800mg/kg of *B.pilosa* extract, a significant high MCH value was noticed on day 14 when compared to days 0 and 28.

Considering the Mean cell haemoglobin concentration(MCHC),it was also noted that amongst the rats that were treated with 400mg/kg and 800mg/kg of *Bidens pilosa* extract a significant increase in value of MCH was recorded on day 14 when compared to days 0 and 28

Amazingly, the red cell distribution width(RDW) significantly($P<0.05$) increased in all treatments groups from day 0 to 28 with exception of rats treated with 400mg/kg of *Bidens pilosa* rats where a reduction in RDW value was noticed on day 28.However the reduction in MCH value was not significant when compared to day 14.

Table 4.1 Result of the phytochemical analysis of aqueous *Bidens pilosa* leaf extract .

Bioactive components	Results
Saponins	++
Tanins	$Fecl_3$ +;Iodine -
Resins	-
Phenol	-
Alkaliods	Picric acid +;Meyers Rgts +
Anthraquinones	+
Steroides	+
Terpens	-
Cardiac glycosides	+++
Carbohydrates	++
Flavonoides	+++
CH_3Coopb	+++
$NAOH_3$	++

Key: - Absent; + Slightly Present;++ Moderately present;+++ Highly present

Table 4.2 Mean body weight and Standard Deviation values of rats(grams)

Treatment group	Day 0	Day 14	Day 28
Control*	107.58±26.36	120.73±20.35	137.35±21.78
200mg/kg	183.62±21.24	172.52±17.11	192.78±15.14
400mg/kg	178.68±23.21	164.17±21.39	169.92±20.91
800mg/kg	163.27±33.39	167.77±35.57	187.65±32.32

*Control is significantly different from the rest of the treatment group at $P<0.05$

Table 4.3 Mean percentage change in body weight and standard deviation values of rats(%)

Treatment group	Day 14 (Percentage)	Day 28 (Percentage)
Control	14.07±10.28[b]	24.66±33.53[c]
200mg/kg	-5.83±4.10	5.42±5.30[a]
400mg/kg	-6.00±22.20	-3.08±20.52
800mg/kg	2.65±1.66	15.52±4.15[a]

[a]Day 14 is significantly different from day 28 at P< 0.05
[b]Control group is significantly different from 200mg/kg and 400mg/kg on day 14
[c]Control group is significantly different from 400mg/kg on day 28

Table 4.4 Mean and Standard Deviation of Haemostatic indices of Wistar rats administered with graded doses of aqueous extract of *B.pilosa* and Control

Parameter	Treatment group			
	Control	200mg/kg	400mg/kg	800mg/kg
PT(sec)				
Day 0	14.16±4.27	10.28±2.36	12.15±2.98	14.36±5.88
Day 14*	13.17±5.60	15.40±4.23	10.17±4.92	17.17±2.71
Day 28	12.34±3.42	16.00±4.00	11.73±4.31	12.97±1.16
APTT(sec)				
Day 0*	20.62±6.37	30.73±15.24	18.79±3.01	32.52±15.26
Day 14	16.83±6.34	22.00±15.17	17.83±9.47	12.17±6.21
Day 28*	17.56±8.74	18.21±6.21	28.94±14.48	14.60±4.40
TT(sec)				
Day 0	15.45±8.36	18.07±7.32	15.77±4.55	19.19±2.57
Day 14	17.00±9.97	13.50±4.04	14.50±4.55	17.60±2.97
Day 28**	15.35±1.54	15.09±1.43	19.72±3.63	17.38±3.06

*400mg/kg is significantly different from 800mg/kg at $p < 0.05$

**400mg/kg is significantly different from control and 200mg/kg at $p < 0.05$

KEY: PT;Prothrombin Time **APTT**;Activated Partial Thromboplastine Time

TT;Thrombin Time

Table 4.5 Mean (±SEM) Haemostatic indices in relation to duration in Wistar rats within treatment group

Parameter	Treatment group											
	Control			200mg/kg			400mg/kg			800mg/kg		
	Day 0	Day14	Day28	Day 0	Day14	Day28	Day 0	Day14	Day28	Day 0	Day14	Day28
PT[a]	14.2±4.3	13.2±5.6	12.3±3.4	10.3±2.4	15.4±4.2	16.0±4.0	12.2±3.0	10.2±4.9	11.7±4.3	14.4±5.9	17.2±2.7	13.0±1.2
PTTK[a]	20.6±6.4	16.8±6.3	17.6±8.7	30.7±15.2	22.0±15.2	18.2±6.2	18.8±3.0	17.8±9.5	28.9±14.5	32.5±15.3[a]	12.2±6.2	14.6±4.4
TT	15.4±8.4	17.0±9.0	15.4±1.5	18.1±7.3	13.5±4.0	15.1±1.4	15.8±4.6	14.5±4.6	19.7±3.6	19.2±2.6	17.6±3.0	17.4±3.1

[a]Day 0 is significantly different from day 14 and 28 at $P<0.05$ for 200mg/kg and 800mg/kg

Table 4.6 The Mean(±STD) of Thrombocyte Indices of Wistar rats administered with graded doses of aqueous extract of *Bidens Pilosa* leaves(BPL) and Control

Treatment group	Parameter			
	PLTx10^9	MPV(fl)	PDW(%)	PCT(%)
Control	392.25±200.76	6.65±0.51	21.93±8.28	0.27±0.16
200mg/kg	421.29±177.37	6.82±0.52	23.43±8.61	0.28±0.16
400mg/kg	463.50±164.36	6.93±0.90	26.40±7.79	0.30±0.15
800mg/kg	522.50±299.22	6.83±0.44	25.49±7.75	0.36±0.22

No significant difference at ($P<0.05$) was recorded in all treatment.
Key; PLT-Platelet count;MPV-Mean Platelet Volume;PDW-Platelet Distribution Width.

PCT-Platetocrit

Table 4.7 Mean(±STD) of Thrombocyte indices in relation to duration in rats within treatment group

Parameter	Treatment											
	Control			200mg/kgl			400mg/kg			800mg/kg		
	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28
PLTx10^9	244.3±72.9[a]	565.7±270.5	514.7±44.4	318.0±136.7	602.5±181.6[c]	379.6±102.3	305.0±173[a]	538.0±179.7	515.8±140.8	242.8±153.2[a]	656.0±285.9	666.2±265.1
MPV(fl)	6.4±0.3[b]	7.2±0.6	6.7±0.5	6.4±.3	6.9±11.2	5.8±4.0	6.3±0.3	7.7±0.9[c]	6.6±0.7	6.4±0.1	7.4±0.2	6.9±0.3
PDW(%)	15.2±0.2[a]	27.3±9.9	30.1±0.4	6.4±0.3[a]	7.0±0.8	7.0±0.1	15.4±0.3[d]	32.9±2.6[d]	28.6±3.2[d]	15.1±0.3[a]	31.5±0.3	30.2±1.8
PCT(%)	0.2±0.04[a]	0.4±0.2	0.3±0.02	0.2±0.1[b]	0.4±0.2	0.3±0.1	0.2±0.04[b]	0.4±0.1	0.3±0.1	0.1±0.09[a]	0.6±0.2	0.5.0±0.2

[a]Day 0 is significantly different from day 14 and 28 at P<0.05 [c]Day 14 is significantly different from day 0 and 28 at P<0.05
[b]Day 0 is significantly different from day 14 at P< 0.05 [d]All the days are significantly different from each other at P<0.05

Table 4.8 Mean(±STD) of Leucocyte indices of Wistar rats administered with graded doses of aqueous extract of *Bidens pilosa* leaves(BPL)and control.

Treatment group	Parameter			
	WBCx10^9	Lymphocyte%	Monocyte%	Granulocyte%
Control	6.13±1.48	68.33±9.50	5.36±3.68	26.33±8.40
200mg/kg	8.49±3.69*	60.65±16.86	7.25±5.72	32.09±18.45
400mg/kg	7.45±2.86	68.71±11.58	9.35±5.28*	21.95±10.05
800mg/kg	7.75±1.96	59.79±11.18	10.32±6.84*	29.91±8.35

*Significantly different from control at $p < 0$

Table 4.8 Mean(±STD) of Leucocyte indicesof Wister rats administered with graded doses of aqueous extract of *Bidens Pilosa* leaves(BPL)and control.There was a significant($P<0.05$) increase in WBC among 200mg/kg group compared to control group.However,there were no significant changes in WBC indices amongst other group

Table 4.9 Mean (±STD) of Leucocyte indices in relation to duration in rats within treatment group

Parameter	Treatment group											
	Control			200mg/kg			400mg/kg			800mg/kg		
	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28
WBCx10^9	6.2±1.8	6.5±1.6	5.6±1.0	7.5±2.6	8.1±1.4	9.7±5.7	7.9±2.2	7.4±2.0	7.1±4.3	7.8±1.5	8.9±2.7	6.8±1.4
Lymph%	74.4±4.7	56.4±10.5[a]	68.1±2.0	55.9±26.5	69.4±11.2	58.4±4.0	72.1±8.5	76.7±6.3	58.0±10.3[c]	69.2±6.7[f]	50.8±10.7	59.5±9.2
Mono%	2.9±0.4[b]	5.8±2.9	9.9±3.9	2.6±0.5[b]	8.3±7.3	11.0±4.5	2.9±0.5[d]	9.5±2.6[d]	14.3±3.4[d]	2.7±0.3[g]	11.6±4.5	15.4±5.9
Gran%	22.7±4.6	37.8±8.1[a]	22.1±2.0	41.5±26.6	22.3±13.7	30.6±7.1	25.0±8.9	13.8±8.4[e]	27.7±8.1	28.1±6.7	37.7±7.4	25.1±6.4

[a]Day 14 is significantly different from day 0 and 28 at P< 0.05

[b]Day 0 is significantly different from day 28 at P< 0.05

[c] Day 28 is significantly different from day 0 and 14 at p = 0.05

[d]All the days are significantly different from each other at p = 0.05

[e]Day 14 is significantly different from day 28 at P<0.05

[f]Day 0 is significantly different from day 14 at P< 0.05

[g]Day 0 is significantly different from day 14 and 28 at P<0.05

Table 4. 10. Mean (±STD)of erythrocyte Indices of Wistar rats administered with graded doses of aqueous extract of *Bidens Pilosa* leaves(BPL)and control.

Treatment group	Parameter						
	RBCx10^{12}	HB(g/L)	HCT(%)	MCV(fl)	MCH(pg)	MCHC(g/l)	RDW(%)
Control	7.62±0.77	144.92±18.68	44.27±3.66	58.23±3.65	19.00±1.67	333.67±36.09	14.93±2.27
200mg/kg	8.19±1.12	149.77±28.50	44.39±6.32	54.71±1.64*	18.47±1.62***	337.92±27.06	15.69±2.45
400mg/kg	7.73±1.21	157.50±29.99	46.06±7.46	59.57±2.93**	20.33±1.60*	341.07±19.59	16.23±1.93
800mg/kg	7.27±0.85	136.31±23.10***	41.33±5.17***	56.92±3.27***	18.66±1.63***	328.08±20.06	15.83±2.58

*Significant from control at P< 0.05
**Significantly different from 2000mg/kg at P<0.05
***Significantly different from 4000mg/kg at P<0.05

Table 4.11 Mean (±STD) of Erythrocyte indices in relation to duration in Wistar rats within treatment group

Parameter	Treatment group											
	Control			200mg/kg			400mg/kg			800mg/kg		
	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28	Day0	Day14	Day28
RBCx10^{12}	7.7±0.5	8.0±1.0	7.0±0.8	8.3±0.6	9.1±0.4[a]	7.4±1.3	7.7±0.9	8.7±0.8a	6.8±1.1	7.2±0.6	8.0±0.9[a]	6.8±0.7
HB(g/L)	138.5±14.3	156.0±28.2	146.7±17.4	135.5±10.5	178.5±14.2[b]	138.2±31.7	142.8±5.9	188.6±16.1[b]	138.2±27.3	123.0±11.5	164.3±18.9[b]	124.6±10.2
HCT(%)	43.3±3.2	46.2±4.6	44.3±4.4	43.5±4.3	50.5±3.4[a]	40.4±6.7	45.4±2.7	52.9±3.6[a]	39.8±7.4	38.5±3.2	46.8±4.7[b]	39.3±3.6
MCV(fl)	56.2±2.4	57.6±2.9	63.0±1.7[c]	53.6±0.9	55.7±2.3	55.0±1.2	59.0±3.0	61.2±2.9	58.4±2.7	53.7±1.1[d]	58.8±3.1	58.0±3.0
MCH(g/L)	17.9±1.5[e]	19.3±1.1	21.0±0.9	17.2±1.2[f]	19.7±1.8	18.5±1.1	18.6±1.4[g]	21.8±0.6[g]	20.3±0.9[g]	17.1±0.5	20.5±1.0[b]	18.4±1.1
MCHC(g/l)	319.0±16.5	335.3±32.3	361.3±60.4	322.0±28.0	353.8±26.6	338.0±23.9	314.5±10.4[d]	356.2±8.9	347.2±6.9	319.5±11.7	349.0±6.8[b]	318.2±20.9
RDW(%)	12.9±0.8[d]	16.4±1.8	17.3±0.6	13.3±1.2[g]	15.8±2.1[g]	18.1±0.7[g]	14.1±1.6[d]	17.5±1.6	16.6±0.7	12.5±1.3[d]	17.2±1.8	17.4±0.5

[a]Day 14 is significantly different from day 28 at $P < 0.05$

[b] Day 14 is significantly different from day0 and 28 at $P<0.05$

[c] Day 28 is significantly different from day0 and 14 at $P< 0.05$

[d] Day 0 is significantly different from day 14 and 28 at $P< 0.05$

[e] Day 0 is significantly different from day 28 at $P<0.05$

[f] Day 0 is significantly different from day14 at $P<0.05$

[g]All the days are significantly different at $P< 0.05$

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The phytochemical screening of *Bidens pilosa* leaf(BPL) extract tested positive for saponins, flavonoids, cardiac glycosides, carbohydrates ,tannins ,steroids, anthraquinones. The extract of *Biden pilosa* is very rich in flavoinods as shown by the studies carried out by (Bartolome *et al.,*2013; Ezeonumelu *et al.,*2011; Sundararajan *et al.,* 2006). It has been reported that polyacetylene and flavoinoids are the main bioactive components of BPL extract. According to findings, polyacetylenes inhibits various pathogenenic organisms while flavonoides reduce inflammations (Sundararajan *et al.,* 2006).Analysis of various species of *Bidens* have been carryout in several countries and studies showed that there were some variation in level of activity of different species of *Bidens.* This was attributed to different levels of active ingredients, however the general property appear similar (Bartolome *et al.,* 2013; Andrade-Nato *et al.*, 2004)These phytochemical components have been found to be responsible for the various medicinal and haemostatic properties of *Bidens pilosa* and these activities include antioxidant and anticoagulation activity due the presence of flavonoides (Brandao *et al*.,1997). In addition flavonoides ,one the bioactives components plays a role in mediating in prostaglandin-synthesis, likely through the inhibition of cyclooxygenase (COX) enzymes .Flavoniodes has been shown to participate in endothelial nitric oxide production which inhibits the adhesion and aggregation of platelets, one of the first steps in blood clot formation. In addition, flavonioides has protective and therapeutic effects on animal liver injury, which might be associated with

its antioxidant properties and inhibition of nuclear factors-kB activation(Li-Ping *et al.*,2008).

The acute toxicity studies are carried out to ascertain the dose that will produce either mortality or serious toxicological effects or high level of safety when given once or over a few administrations. They also serve to provide information regarding doses that should be used in sub-chronic or chronic studies.

Acute toxicity tests showed that extract can be considered to be virtually safe since it did not cause death in rats and no signs of toxicity such as alteration of skin, sides effects on gastrointestinal system and general signs of toxicity were not observed after 72 hours. The results of this study confirms the previous work of Adedapo *et al.*,2011 that *B.pilosa* leaf has low level of toxicants. In sub-chronic toxicity studies, there was no mortality throughout the duration of the experiment thus showing high safety index of BPL in rats. Increase in body weight was recorded along each group from day 14 to day 28 contrarily to increase from day zero to day 28 reported by (Ezeonumelu *et al.,*2011). However, this increase in body weight could be attributed to the nutritive value of *Biden pilosa* pant as reported by (Adedapo *et al*.,2011; Duke,1997)

Also a general significant increase at $P<0.05$ in percentage body weight gain was recorded between day 14 and day 28 along each group. Down the group, significant reduction($P<0.05$) in percentage weight gain was observed on day 14 between control group(14.07g),200mg/kg group(-5.83g) and 400mg/kg group(-6.00g).Interestingly, on the same day 14, a significant increase($P<0.05$) in percentage body weight gain was also observed between 400mg/kg group(-0.06g) and 800mg/kg group(2.65g).The observed reduction in body weight gain among rats administered with *Bidens pilosa* down the groups was somewhat dose dependant. This could be related to the amount of *B.pilosa*

active chemicals which have positive or negative effects on some parameters This pattern of reduction in weight gain caused by *Bidens pilosa* is in line with the findings of (Duke,1997) that the plant could be used as a weight loss aid.

Haemosatasis which is the arrest of blood loss from severed vessels and maintenance of blood fluidity involves coagulation and fibrinolysis. Once a blood vessel is severed, it leads to vasoconstriction and thrombin activation which are then accompanied by platelets activation, adhesion and aggregation (Azikiwe *et al*.,2007). The prothrombin time (PT) was developed to measure Prothrombin (Factor II) and hence its name. However, it subsequently became clear that it was sensitive to abnormalities of factors VII, X, V, II and fibrinogen. The Activated Partial Thromboplastin Time Test (APTT) in contrast to the PT, measures the activity of the intrinsic and common pathways of coagulation. In the present study, result showed that the extract of *Biden pilosa* significantly ($P<0.05$) increased prothrombin time (PT) on day 14 amongst rats treated with 800mg/kg compared to 400mg/kg group where a significant reduction of PT was noticed.This indicates that *Bidens pilosa* extract has the potential to cause changes to extrinsic factors when administered at higher dose. This could be associated with anticoagulation activities of the *Bidens pilosa* extract. However it was observed that *Bidens pilosa* extract significantly ($P<0.05$) decreased APTT on day 28 among the 800mg/kg group compared to 400mg/kg group. This could be attributed to increased activities of intrinsic factors and coagulation properties of *Bidens pilosa* extract. This could be ascribed to hepatoprotective role of *Bidens pilosa* (Chin,*et al*., 1996).There was a significant($P<0.05$) increase in Thrombin Time(TT) at dose 400mg/kg and 800mg/kg when compared with control and 200mg/kg groups. The increase could be attributed to

decreased activity of *Bidens pilosa* extract on common pathway such as factors I,II and XII

Effect of BPL on haemostatic activities within each treatment group with respect to duration(Table 4.5) also indicates a significant increase($P<0.05$) in prothrombin time values among rats that received 200mg/kg of extract on day 14 and 28 when compared to day 0 .Also, rats that received 800mg/kg b.w. had a significant increase in PT on day 14 when compared to days 0 and 28.This is an indication that the extract of *B.pilosa* may have suppressed one or more of the clotting factors involved in extrinsic pathway. The effect of aqueous *BIdens pilosa on* thrombin time follows the same pattern with that of PT. So this signifies that *Bidens pilosa* extract has some anticoagulation properties with respect to PT assay in rats that received 200mg/dl and 800mg/dl b.w.

Also a significant decrease($P<0.05$) in APTT values recorded especially on day 14 among all the groups that received *Bidens pilosa* extract suggest the procoagulation property of the aqueous extract. This reveals that there could be an increase in one or more of the clotting factors involve in intrinsic factors This finding is in consonance with the previous studies on the effects of tropical plants extracts; According to Tanko *et al.,*(2012),it was revealed that Mushroom((*Ganoderma lucidum*) reduced the clotting time in rats. In addition, *Ageratum conyzoides* has shown to significantly reduce bleeding and clotting times in albino wister rats (Bamidele *et al*.,2010),while, *Aspilia Africana* has shown to arrest bleeding from fresh wounds by reducing bleeding and clotting times(Okoli *et al.*,2007). This is in line with the use of sap from crushed leaves of this *Bidens pilosa* plant to speed up clotting of blood in fresh wounds in Plateau State, Nigeria. However, haemostatic activities of BPL are both dose and dosage dependant. This to say that the

haemostatic indices of rats that received *Bidens pilosa* extract depends on the quantity and duration it was administered.

The significant($P<0.05$) increase in platelets counts with increase in dosage of BPL extract in rats recorded(Table 4.7) suggests that the aqueous extract of BPL has some thrombocytic activities in rats. Despite the increase in platelets among rats that received the high dose of BPL extract, it has been shown that the extract of BPL lowers blood pressure in rats, potentially through its vasodilating activity. So BPL could be used as an antiaggregant in prevention of thrombotic related diseases (Zang,1989)..

A significant($P<0.05$) increase in WBC observed only among the 200mg/kg group compared to control, and for other leucocytes indices, no significant change in concentration was noticed among the groups . Table 4.9 indicates the effect of BPL on leucocytes indices in relation to duration within each treatment.WBC showed no significant difference in all the groups with respect to duration. However, there was a significant ($P<0.05$) decrease in the percentage of lymphocytes among the control groups on day 14 when compared with day 0 and day 28.For among the 400mg/kg group a significant($P<0.05$) decrease in percentage of lymphocytes was observed only on day 28 when compared with days 0 and 14.While among the 800mg/kg group a significant($P<0.05$) decrease in lymphocytes percentage was noticed on days 14 and 28 when compared with day 0. This is an indication that aqueous extract of BPL is dose and dosage dependant .In addition the decreased in percentage of lymphocytes observed, it is pertinent to note that the PBL extract may mediates in regulation of concentration of Lymphocyte with respect to duration. This is in line with previous studies carried out by Pereira *et al* (1999,) suggesting that extract of BPL, has some immunosuppressive activity in mice. In addition it has the potential to regulate the host immune response to type 1

allergy. Therefore BPL extract or its flavonoides content may suppress the production of IgE by improving the T cell balance (Masko *et al*., 2006).This is in line with in-vivo and in-vitro studies that revealed that methanol extract of BPL suppressed human lymphocyte proliferation (Pererira *et al*.1999).

The result also revealed a significant ($P<0.05$) increase in monocytes in rats that received 400mg/kg and 800mg/kg of aqueous extract BPL when compared with control group. A study that used hydroalcoholic extract of *B.pilosa* found a predominace of Mononuclear cells in rats (Lermen *et al.,*2012).This could be attributed to the role of BPL extract plays in leucocyte mobilization. However there were no significant change in granuloytes in the study group compared to control groups.

Evaluation of haematological parameters can be used to determine the extent of the deleterious effect of *Bidens pilosa* extract on the blood of an animal. It can also be used to explain blood relating functions of a plant extract or its products(Yakubu *et al.,*2007). Furthermore, such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies(Olson,2000) The results of effects of *Bidens pilosa* on erythrocytic indices showed that the Haematocrit (HCT) values, haemoglobin (HB) concentrations, Mean cell Voume (MCV) and Mean cell concentration (MCH) values were lowest among groups of rats treated with 800mg/kg of extract compared to controls groups. This is an indication of deleterious effect *Bidens pilosa* at high dose and should be given with caution. Haemoglobin concentration is used to determine the oxygen carrying capacity of blood while the HCT determination indicates the relative mass of red cell in a sample of blood (Lewis, 2006).Also the MCV and MCH indices are usually important in elucidating and classifying anemia morphologically. The results of this study revealed the effect of extract

is dose dependant and emphasized on blood relating functions of a plant extract. That is to say that at higher dose the effect could be deleterious to some hematological parameters despite the property to protect normal erythrocytes against oxidative damage *in vivo (*Yang,et *al.,*2006).

The significant increase in concentration of RBC,HCT ,HB ,MCV,MCHC and RDW values with respect to duration within treatment group on day14(Table 4.11) is an indication that the extract of BPL may have some haempoietic property. This is an indication that there was no destruction of matured RBCôs and there were changes in the rate of production of RBCs (erythropoiesis). It further shows that the extract t have the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production .The significant effect on the RBC ,HB also implies that there was change in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following the extract administration on day 14 since RBC and Hb are very important in transferring respiratory gases

This trend of increase is comparable with that reported by Ihedioha *et al* (2004) on the haematological profile of the Sprague-Dawley Outbred Albino rat in Nsukka.However according to research carried out by Yang,et *al.,*(2006) it was discovered that extracts from the whole *Bidens pilosa* plant have the property to protect normal human erythrocytes against oxidative damage *in vitro.*

5.2 CONCLUSION

In conclusion, the overall result of this study clearly demonstrate that oral administration of aqueous *Bidens pilosa* leaves extract leads to slight haemostatic and haematological changes in rats despite the high safety profile index recorded in acute and sub-acute toxicity test.

5.3 RECOMMENDATIONS

1 A comparative study of different extract of *Bidens pilosa* on haemostatic and haematological profile should be carried out to compare the level of bioactive molecules using different methods of extraction.

2 A profile of bioactive ingredients of *Bidens pilosa* should be carried out to ascertain the bioactive molecules of the *Bidens pilosa* extract that exerts the haemostatic and haematological changes recorded in rats.

5.4 CHALLENGES

1. The issue of financial constraint was a big challenge
2. Taking care of the animals adequately was actually demanding.

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