SOME PHARMACOLOGICAL STUDIES ON THE ETHANOLIC STEM BARK EXTRACT OF *PTEROCARPUS ERINACEUS* POIR (FABACEAE)

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DECLARATION.

I declare that the work in this thesis entitled SOME PHARMACOLOGICAL STUDIES ON THE ETHANOLIC STEM BARK EXTRACT OF *PTEROCARPUS ERINACEUS* (FABACEAE) has been performed by me in the Department of PHARMACOLOGY AND CLINICAL PHARMACY under the supervision of Drs (Mrs) A.O. Salawu and J.A. Anuka.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any university.

Aliyu Musa June, 2006

CERTIFICATION

This thesis entitled “SOME PHARMACOLOGICAL STUDIES ON THE ETHANOLIC STEM BARK EXTRACT OF *PTEROCARPUS ERINACEUS* POIR (FABACEAE)” by Aliyu Musa meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Some pharmacological effects of the 70% ethanolic stem bark extract of *Pterocarpus erinaceus* were investigated in experimental animals.

Preliminary Phytochemical studies of the ethanolic extract revealed the presence of Tannins, Carbohydrates, Proteins & Amino acids, Flavonoids and Steroids.

Acute toxicity test using Lorke's method indicated LD_{50} values of the ethanolic stem bark extract of *P.erinaceus* to be 447.21mg/kg I.P and >5000mg/kg *P.o* in rats.

In the sub acute toxicity studies, haematological parameters like Haemoglobin concentration (Hb), packed cell volume (PCV), Platelet count, White blood cell count (WBC) and differential count of WBC were within normal limits.

A significant decrease ($P<0.05$) in bleeding time was observed in rats treated with 200mg/kg extract. The clotting time only slightly decreased.

The extract *P.o* did not cause any death during the period (30 days) of administration.

The ethanolic stem bark extract of *P.erinaceus* at doses 50,100 and 200mg/kg body weight caused significant ($P<0.05$) dose dependent antinociceptive activity in both (central and peripheral) pain models used. The peripheral action may be linked partly to lipooxygenase and / or cyclo-oxygenase, while the central antinociception is probably mediated via opioid receptors in the CNS.

The ethanolic stem bark extract produced significant ($P<0.01$-0.001) dose dependent anti-inflammatory effects against egg-albumin induced inflammation in rats. The effect of the extract was however lower than the standard drug used piroxicam.

The ethanolic stem bark extract of *P.erinaceus* relaxed the isolated pregnant rat uterus. Oxytocin induced contractions of the pregnant rat uterus was blocked by the extract dose dependently.

The studies on isolated guinea- pig ileum and rabbit jejunum smooth muscles demonstrated that the ethanolic stem bark extract of *P.erinaceus* produced a dose dependent relaxation these muscles. The extract also attenuated the contractile effects of acetyl choline on these tissues dose dependently. This finding might lend credence to the use of the stem bark of the plant in the treatment of diarrhea and dysentery traditionally.

From the results of this work and information from literature, flavonoids and tannins identified during phytochemical screening of the extract may be the biologically active principles responsible for the haemostatic, anti-nociceptive, anti-inflammatory and gastrointestinal effects of the ethanolic stem bark extract of *P.erinaceus.*

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LIST OF ABBREVIATIONS

Ach	-	Acetylcholine
AIDs	_	Acquired immune deficiency syndrome
C^{++}	_	Calcium ions
$CaCl_2$	_	Calcium chloride
c-AMP	_	Cyclic adenosine monophosphate
cm	_	Centimeter
CNS	_	Central nervous system
COX	_	Cyclooxygenase
EDTA	_	Ethylediethyl tetra acetate
e.g	_	exempli gratia (for example)
Et al	_	et alli (and others)
e.t.c	_	et cetera (and so on)
Fig.	_	Figure
g	_	grams
Hist	_	histamine
hr	_	Hour
i.e	_	id est (that is)
IUDS	_	Intra Uterine Device
IP	_	Intraperitoneally
Kg	_	Kilogram
KCl	_	potassium chloride

L	_	Liters
LD_{50}	_	Lethal dose 50
M	_	Meters
mg	_	milligrams
min	_	minutes
ml	_	milliliter
PG	_	Prostaglandins
P.e	_	*Pterocarpus erinaceus*
P.o	_	oral
Sec	_	seconds
U.S.P	_	United States Pharmacopoiea
µg	_	micrograms
α	_	Alpha
β	_	beta

Chapter 1

INTRODUCTION

Nature has always been the mother of all treatments, which provides therapies for all illnesses and diseases.

Through the process of studying nature, man studied plants, some of which he found to be useful for food and cultivated same as food crops, while those he found harmful or poisonous were rejected. He found another group of plants to possess medicinal properties, used them as such and the special knowledge passed on to his progeny (Chame, 1987).

Traditional medicine is the oldest, most tried and tested form of medicine and is as old as man himself.

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO,2000).

Traditional medication involves the use of herbal medicines, animal parts and minerals. As herbal medicines are the most widely used of the three, and as the

other types involve other complex factors, emphasis is now focused on traditional medicines.

Practices of traditional medicine vary greatly from country to country, and from region to region as they are influenced by factors such as cultures, history, personal attitudes and philosophy.

World Health Organization (WHO, 2003) has estimated that perhaps 80% of more than 400 million inhabitants of the world rely mainly on traditional medicine for their primary health care needs (Akerele, 1990). There is an increasing use and popularity for traditional medicine throughout the world nowadays. In industrialized countries, adaptation of traditional medicine is termed "Complementary" or "Alternative" medicine (CAM).

In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60 percent of children with high fever resulting from malaria is the use of herbal medicine at home (WHO, 2003).

In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use traditional medicine (WHO, 2003). Traditional medicine also has impact on infectious diseases. For example, the Chinese herbal remedy *Artemisia annua*, is already in use against resistant malaria and has created a breakthrough in preventing almost one million deaths annually, most of them children, from severe fever (WHO, 2003).

It is estimated that 25% of modern medicines are made from plants first used traditionally (WHO, 2003).

Long historical uses of many traditional medicines, including experience passed on from generation to generation have demonstrated the safety and efficacy of traditional medicine. However, scientific research is needed to prove evidence of safety and efficacy (WHO, 2000).

1.1 Medicinal Plants

Plants represent the principal means of therapy in traditional medicine and the plant kingdom has long served as a prolific source of useful drugs.

A medicinal plant could be defined as any plant which in one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical synthesis.

A lot of postulations have been suggested as to how the medicinal uses of plants were revealed to man.

Paracelsus brought the “Doctrine of signature”. It was believed that benefits are manifested in a sign (or signature) on the plant to indicate their uses to man. A liver shaped leaf indicated the plants used in liver diseases, while a heart shaped leaf indicated cardiac activities (Kafaru, 1994).

A postulation that plants grow according to the needs of mankind also came in. It was believed that new plants that grow around the home often ought to be plants needed by someone who is sick in the house (Kafaru, 1994).

It was also believed that animals wounded by other animals or hunters run to the forest to eat some particular type of plants, such plants were then marked as medicinal.

A great number of efficacious plant extracts appear to be available for the alleviation of many disorders.

The treatment of glaucoma with alkaloid from the Calabar bean (*Physostigma venenosum*) of Nigeria or leaves of Brazillian species of *pibocerpus,* can prevent blindness. *Physostigma erythrophyllum* is found to relieve pressure on neural receptor sites in that organ (Lely, 1958).

The discovery of antineoplastic agents Vincristine and Vinblastine alkaloids of Madagascan annual periwinkle in treating Hogkin's disease, acute lymphocytic leukemia, Wilm's tumor, Burkkitt's lymphoma and gestational chorio-carcinoma is also a milestone in medicine.

Classical examples of the debt medicine owes to plants include use of quinine, morphine, cocaine, reserpine, emetine and atropine.

Efforts are continuously being made to identify the rationale behind the use of medicinal herbs. Such efforts include:

a) Use of chewing sticks in cleaning teeth - in many African homes; teeth are cleaned in the morning by chewing the root or slim stem of certain plants until they acquire brush like ends (El-Said *et al.,* 1970). The fibrous end is then used to brush the teeth thoroughly. On scientific evaluation they were shown to possess varying degrees of anti-microbial activity against oral microbial flora. Plants used for this purpose include *Zanthoxylum zanthoxyloides, Terminalia glancescens, Anogeissus leiocarpus, Vernonia amygdalina and Garcinia kola*.

b) Use of *Ocimum gratissimum* in treating diarrhoea.

Ocimum gratissimum leaf or whole herb is a popular treatment for diarrhoea (Dalziel, 1956). The plant is rich in volatile oils, which contain up to 70% of thymol, an antimicrobial.

However, in certain preparations, *O. gratissium* is boiled with water to form a decoction that will contain little of the steam volatile thymol. Such aqueous decoctions were shown to be devoid of anti-microbial activity, but they do relax the guinea pig ileum and rat jejunum in vitro.

c) The use of neem (*Azadirachta indica*) to treat malaria.

Hedges of the neem tree are grown close to houses because this plant is highly esteemed as a fever cure especially in malaria fever, which is endemic in Africa. A decoction of the leaves, or of leaves and stem bark, is drunk while in some cases the stem bark is also used to treat fevers by inhalation or hydrotherapy.

Ekanem, (1978) reported a fall in parasite count in chloroquine-sensitive strains of *Plasmodium berghe*i infected mice treated with a decoction of the leaves of *Azadirachta indica*. Ade-Serrano (1982) also reported the growth inhibitory effect of the leaf extract on P. falciparum culture while Okpanyi (1977) showed its anti-inflammatory property. Okpanyi and Ezeuwor (1981) demonstrated that an extract of the leaves and bark produced antipyretic effect, thus justifying its use in fever.

Others include the use of *Montanoa tomentosa* as a contraceptive, *Rauwolfia vomitoria* in treating lunatics and *Bridelia ferniginer* in treating diabetes.

The chemical constituents in medicinal plants usually explain the rational for the use of the plant in traditional medicine. The trend now is that phytochemists exploit medicinal plants and isolate bioactive compounds from which different analogues are synthesized with the aim of obtaining agents with better actions or

even different biological properties. Plant's active constituents thus serve as templates for future drug developments.

Medicinal importance of plants has been attributed to the presence of their metabolic products (phytochemical compounds). Ordinarily it is these phytochemical compounds that cure diseased plant itself, and are believed to activate, catalyze or initiate same curative reactions in humans.

These phytochemical compounds can be concentrated in any part of the plant like the leaves, roots, bark etc. These compounds include alkaloids, flavonoids, tannins, terpenoids etc, which are responsible for the pharmacological activity of the plant.

1.2. ***Pterocarpus erinaceus (Poir)***

Family: *Leguminosae*

Sub-family: *Papilionoideae, fabaceae*

Pterocarpus erinaceus is a perennial tree that is popularly known as African rosewood, African kino, or teak (in English). *"Pterocarpus"* means 'winged fruits' from the Greek *"Pteron"* (Wing) and"Karpos" (fruit).

It is a deciduous legume tree of African Savannas and dry forest famous for producing one of the finest woods in its native region. It also produces leafy fodder high in protein, which makes an excellent animal feed crucial for survival of livestock during the dry season. The tree produces showy and attractive golden-yellow flowers and has considerable potential as an ornament. Increasing demand for the high value timber and fodder threatens existing natural strands. However, it is easy to propagate, making it a good candidate for reaforestation programs. In northern Nigeria it is called "madobiya", "sha jinni"," banuhi", "zanchi" while in the south, it is called "apepe", "era", "osun dud" or "upeka".The Ghanaians call it "senyo", bunernga" or "doti". The people of Ivory Coast refer to it as"modia baka" or "tolo", while it is reffered to as "tem", or "butumbu" by theTogolese

Fig.1.1 *Pterocarpus erinaceus tree* in Burun-burun forest,Tudun-dawa, Kano, Nigeria (during dry season).

1.2.1 *Botanical Features of Pterocarpus erinaceus*

Pterocarpus erinaceus poir (fabaceae) is a small to medium size tree that is 12-15m tall with a diameter of 1.2 – 1.8m. In the drier part of its range, it has an open, spreading form and is low branching. Under favourable rainfall and soil conditions, much larger specimens with clean straight botes 6-8m long or more can be found (Lely, 1925). Exceptionally tall trees reaching 35m in height have been reported (Von Maydell, 1983). The bark of the trunk is dark grey and rough, with scales that curl up at the ends. Its branches are light gray and smooth. The leaves are compound, imparipinnate, and 30cm long.

There are 10-15 alternate or sub-opposite leaflets, 6-11cm long and 3-6cm wide (Hutchinson et al, 1958, Lely, 1925). The flowering tree is showy and very attractive, with masses of golden-yellow flowers that completely cover the canopy.

In its native range, *Pterocarpus erinaceus* flowers from December to February (ICRAF, 1998). The fruit is 4-7cm in diameter, indehiscent, and broadly winged, giving it a 'flying saucer' appearance.

The young fruits are light green and turn light brown when dry. The seeds are kidney-shaped to oblong.

Pterocarpus erinaceus has been referred to as *Pterocarpus angolensis* DC and *Pterocarpus echinatus* DC.

1.2.2 *Distribution of Pterocarpus erinaceus*

Pterocarpus erinaceus is found throughout West and Central Africa, ranging from Senegal in the west to the Central African Republic in the east. It is distributed up to 14”N but is a stunted, small tree at this latitude, where another species, P*terocarpus lucens*, takes over and become more abundant. Southward, the range extends to the limit of the humid forest in Cote d-Ivoire and the humid coastal savannas in Guinea, Togo, and Benin, where a gallery-forest species, *Pterocarpus santalinoides*, is common along rivers and temporary watercourses.

1.2.3 *Uses of Pterocarpus erinaceus*

Pterocarpus erinaceus has been used commercially in wood and wood products.It provides some of the finest wood from dry forests of the region. The wood is beautiful, rich rose-red or dark brown, mottled with dark streaks (NAS, 1979). It has handsome, fine-grained appearance and is used for furniture (including stools and benches), decorative paneling, and parquet flooring. It is also used as construction wood and in carpentry, for doors and windows frames. It makes very good charcoal, which is highly valued by local blacksmiths.

The sap dries to a red resin known as Kino, a name of Mandingo origin which when crushed is applied with a mallet to cloth glazing in West Africa. It is also employed as tannin astringent, and in India, as a dye for cotton goods. The roots (preferably), bits of old stem, or stripped bark, are sold in local markets as a dye wood or may be offered in the form of balls made up with palm oil (Hausa -majigi).

Pterocarpus erinaceus provides high quality leaf fodder with an average nutritive value in dry matter of 5.3 MJ Kg^{-1} net energy, 16-19% crude protein, and 0.15% phophorus. Pastoralists traditionally lopped wild trees in silvopastoral systems to feed their livestock during the dry season. Increasingly, this fodder is brought to urban and semi-urban markets for sale. In Bamako, more than 1400 tons of fresh *Pterocarpus* leaves are sold annually to feed the estimated 11,000 sheep in the city (Anderson et al 1994). Because of the huge demand, the tree is so heavily lopped that it is now difficult to find in the periphery of Bamako, where it used to be readily available. Vendors of *Pterocarpus* fodder are now forced to travel up to 50 km to find trees that still have leaves to harvest (ICRAF 1997, Bonkoungou et al. 1998).

In traditional medical practice, the leaf infusion is used in Ghana for fever (Irvine, 1961). The bark and resin decoction is an astringent for severe diarrhea and dysentery. The bark decoction has also been used for the treatment of tumors of the gland, urethral discharges and as restorative. The bark, as a dressing, is used on ringworm of the scalp and chronic ulcers (Dalziel, 1948). The leaf and stem decoction is used as a febrifuge in Ivory Coast (Karharw, 1950).

In Northern Nigeria, the bark is used as an ingredient in abortifacient prescriptions (Dalziel, 1948). In some parts of Kano, some herbalists use it to stop nose bleeding and menorrhagia (Khamis Sangarib, Verbal Communication,).

CHAPTER 2

LITERATURE REVIEW

2.1 Haemorrhage and Haemostasis

Haemorrhage is characterized by arterial, venous or capillary discharge of blood. Many factors may interfere with the normal clotting mechanism and result in various degrees of hemorrhage. These include liver disease, chemical poisoning (heparin, dicumarol), estrogen therapy, exposure to various venoms, coagulation disorders (hemophilia, thrombocytopenia), anaphylaxis, and vascular disorders. Uncontrolled hemorrhage may cause hypotension and hypoxia.

Haemostasis is the arrest of blood loss from damaged blood vessels and is essential to life. It involves three main processes; I) vascular contraction, ii) adhesion and activation of platelets, iii) fibrin formation (blood coagulation),

The last two processes result in the formation of a haemostatic plug that blocks the breach in the vessel and stops the bleeding.

The integrity of the capillaries largely depends on that of the endothelial cells and the cement substance that links them together. They also derive some support from the surrounding tissues.

The capillaries and larger vessels react to injury by an immediate local temporary vasoconstriction that is thought to be due to reflex nervous mechanism. This reduces the

amount of blood lost and the resultant slowing of the blood flow assists in the aggregation of platelets, a process that helps to seal the ends of injured vessels.

The platelets also release substances (e.g adenosine diphosphate (ADP), serotonin, histamine, platelet factor-3 and phospholipids) which initiate coagulation and also play a part at all stages of coagulation process.

Coagulation results in the production of a fibrin clot, which, by subsequent organization and recanalisation, leads to the final healing, and restitution of the injured area (Chesterman *et al.,* 1982).

The blood vessel response, platelet adhesion and aggregation and release of chemical factors are initiated within seconds following vascular injury.

The functional integrity of the initial phase of hemostasis is gauged by the bleeding time test, which may be indicative of pathological changes in vasculature (wall of the capillaries) or of the number and activity of circulating platelets. However, this test has no value in estimating the activity of blood clotting factors.

2.1.1 *Coagulation of Blood*

Control of bleeding is an integrated mechanism. The initial step resides in the vessels and platelets whereas coagulation is a late stage, constituting a third phase of defense against hemorrhage (Quick, 1970).

Coagulation, or blood clotting whether intravascular or extra vascular, involves the interactions of several plasma proteins known as coagulation factors.

The clotting mechanism involves intrinsic and extrinsic pathways. In the intrinsic pathway, upon injury to tissue, the contact activation between shed blood and vascular surface converts inactive factor XII (Hageman factor) to XIIa. This in turn converts XI to XIa, etc. In the extrinsic pathway, tissue factors released interact with factor VII and the product of this step converts factor X to Xa. Both the intrinsic and extrinsic pathways lead to activation of the common pathway, in which factors Xa, V and phospholipids interact to form thromboplastin (stage I coagulation), which supplies activated thromboplastin (enzyme).

In stage II, thromboplastin in the presence of Ca^{++} converts prothrombin (produced in the liver) to thrombin (proteolytic enzyme). With entry into stage III, thrombin activates the soluble protein, fibrinogen (also produced in the liver), into a monomer of fibrin (soluble). Fibrin-stabilizing factor (XIII) converts loose fibrin to a dense, tightly aggregated fibrin by forming covalent cross – linkage between fibrin monomers. The

threads of fibrin, bound together with the platelet thrombus, form the strong, insoluble fibrin clot, which terminates the events of the hemostasis phenomenon. (Fig.2.1)

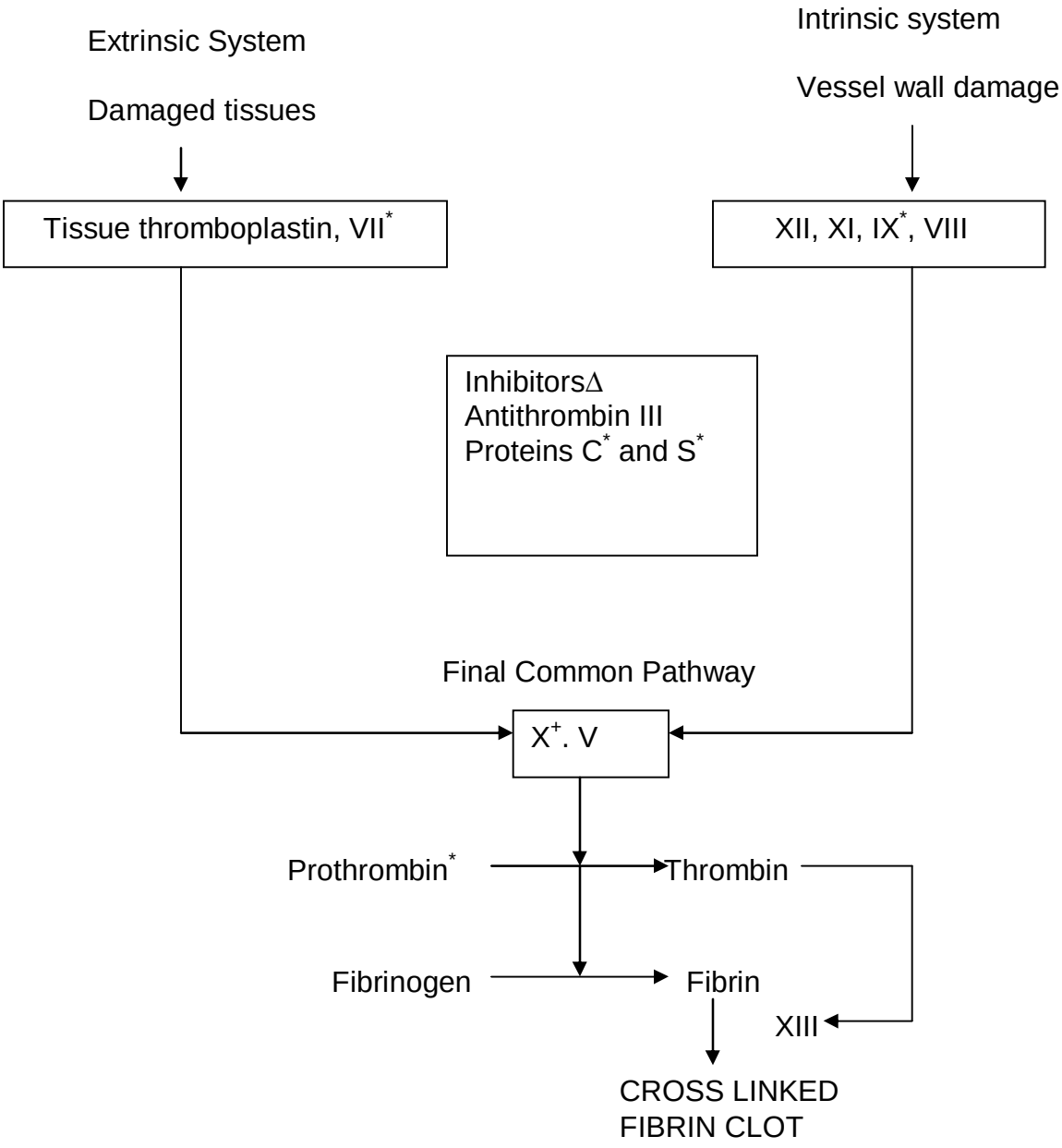

Figure2.1: Schematic Representation of Blood Coagulation System

* Vitamin K dependent

Δ Antithrombin III inhibits IX, X, XI, XII and thrombin
Proteins C and S inhibits V and VIII

The coagulation factors in the plasma are represented as follows;

I —— Fibrinogen

II—— Prothrombin

III—— Tissue thromboplastin

IV—— Calcium

V—— Plasma acceleration globulin, proaccelerin

VII— Proconvertin

VIII— Antihaemophilic globulin (A)

IX — Antihaemophilic globulin (B), Christmas factor

X — Stuart-power factor

XI — Plasma thromboplastin antecedent (PTA)

Antihaemophilic factor C

XII— Hageman factor (contact factor)

XIII— Laki-brand factor, fibrin- stabilizing factor

2.1.2. *Haemostatics.*

These are agents that arrest blood loss from damaged blood vessels either by hastening blood clotting or by acting on the blood vessels. Prototypes of homeostatic agents are subdivided into topical and systemic agents.

Topical haemostatics include thromboplastin U.S.P., fibrinogen U.S.P., fibrin foam, absorbable gelatin sponge U.S.P, oxidized cellulose U.S.P., epinephrine U.S.P. and norepinephrine, U.S.P. Local haemostatic like ferric chloride, ferric sulphate, alum, tannic acid, silver nitrate, zinc chloride and a variety of other astringent substances are also used but with special care as they may damage tissues.

Systemic haemostatic are substances that have coagulating properties. They include:

1) Normal blood: - fresh serum is indicated for emergency treatment in cases of acute hemorrhagic syndromes due to deficiency of clotting factors and thrombocytopenia.

2) Vitamin K: - Vitamin K is essential for the formation of prothrombin and factors VII, IX, and X by the liver. Deficiency of Vitamin K may result in decrease in levels of these coagulation factors and is characterized by hemorrhage into tissue following minor injuries.

Others include ergonovine maleate (Ergonic), carbazochrome salicylate and protamine sulphate.

2.1.3 *Dysfunctional Uterine Bleeding*

Abnormal uterine bleeding includes excessive bleeding, irregular bleeding and absence of bleeding. In about 25 percent of patients, these menstrual irregularities are due to organic causes such as systemic disease, pregnancy, or cancer. For the remainder of patients, there is absolutely no organic pathologic condition but rather a functional abnormality in the hypothalamic-pituitary-ovarian axis defined as dysfunctional uterine bleeding (DUB).

Menorrhagia is a heavy or prolonged menstrual bleeding that may occur as a single episode or on a chronic basis. Normal menstrual flow lasts about 5 days and produces a total blood loss of 60-250ml. In menorrhagia the menstrual period is extended and total blood loss can range from 80ml to overt hemorrhage.

Hypomenorrhea_is an unusually light menstrual flow sometimes only spotting.

Polymenorrhea describes menstruation that occurs too frequently.

Oligomenorrhea_is an abnormally infrequent menstrual bleeding characterized by 3-6 menstrual cycles per year. When menstrual bleeding does occur, it can be profuse and prolonged or decreased in amount.

Amenorrhea (Secondary) is the absence of a menstrual period for 3 or more months in women with past menses precluding physiological causes such as pregnancy, lactation and menopause.

Possible organic causes of abnormal uterine bleeding include, endometriosis, polycystic ovary syndrome, blood dyscrasias, thyroid dysfunction, pelvic inflammatory disease, anorexia nervosa, diabetes mellitus, pituitary disorders, uterine fibroids, cervical stenosis, cervicitis, endometrial polyps, gynecologic carcinoma, syphilis, vaginal adenoids, adrenal disorders and corpus luteum cysts. The use of oral contraceptives (as well as their

discontinuance), anticoagulants, corticosteroids and intrauterine devices (IUDs) can also cause DUB.

2.1.4 *Etiology of Dysfunctional Uterine Bleeding (DUB***)**

Dysfunctional uterine bleeding occurs most commonly at extremes of reproductive age, with 20 percent of cases in adolescence and greater than 50percent in patients over age 40. Normal endometrial bleeding (menstruation) occurs as a result of stimulation of the endometrium by the physiologic levels and balance of estrogen and progesterone present in the normal ovulatory cycle and by the subsequent rapid withdrawal of these two hormones. This withdrawal results in complete and rapid shedding of the entire functional layer of the endometrium.

Various disturbances in this balanced estrogen-progesterone relationship can result in the following four clinical etiologies of true DUB:

i) Non-ovulatory DUB,

ii) Irregular ripening of the endometrium (luteal phase defect)

iii) Irregular (or prolonged) shedding of the endometrium and

iv) Endometrial atrophy.

Obesity and excess adipose tissue in relation to lean body mass also affect estrogen/progesterone ratios.

Anemia contributes to menstrual irregularities. At any level of anemia, the impaired oxygen and nutrient carrying capacity can have dramatic effect on tissues throughout the

body especially reproductive tissues whose functions are dependent upon hormonal messages communicated from the neuroendocrine system through the blood to the target tissues.

Additional causes of anemia can also be nutritional in origin from insufficient dietary iron, Vitamin B_{12}, folic acid, Vitamin C, Vitamin B_6 and Vitamin E, all of which play a role in the uptake and metabolism of iron and formation of healthy red blood cells.

2.1.5 *Use of Selected Herbs in the Treatment of DUB*

Traditional herbal strategies that help regulate excess menstrual blood flow combine plants that (i) help reduce excessive bleeding quickly; (ii) support the functional integrity of uterine tissues and blood vessels (capillaries); (iii) balance female hormones to ensure the normal maturation of ovarian follicle, corpus luteum, and endometrium, (iv) relax muscles; and (v) control inflammation (Joseph ,1997).

Shepherd's purse *(Capsella bursapastoris)* and agrimony (*agrimonia pilosula*) are known for their ability to help control bleeding. In China and Europe, agrimony is used extensively to reduce bleeding and treat profuse menstruation. Agrimony contains tannins, saponins and bitter principles, which can increase coagulation of the blood by up to 50% (Bensky, 1993).

Shepherd's purse classified as an anti-hemorrhagic and urinary antiseptic contain rutin, potassium salts and Vitamin C. In Europe and Germany, it is used in mild menorrhagia and metrorrhagia (Wicht *et al.*, 1994).

2.2. Pain and Analgesia

Pain is a subjective, unpleasant sensory experience. When there is a mechanical, thermal or chemical stimulus, pain sensations are transmitted to the brain, where they are interpreted and felt at nerve endings as pain. Communicating the impulse from the injured part of the body to the brain is done electrically due to a changed distribution of ions across the synapses (discrete gaps between nerves). These are reinforced by the involvement of limbic structures, which are important in emotional behaviour. Pain interpretation is therefore not strictly a sensory event or a protective mechanism for alerting the individual against tissue damage; rather, pain perception is modified based on personal, attitudinal and emotional factors. The resulting impulse is transmitted to the cortical centers of the brain and analyzed as pain (Traynor & Johnson.,1984).

2.2.1. *The Pathophysiology of Pain*

Most definitions of pain do not mention memory, although the memory of pain often overshadows its primary experience in its impact upon pathophysiology and human suffering. Consequently, clinical interventions to blunt both the experience and persistence of pain or to lessen its memory are now applied worldwide (Carr, 1998).

In spite of tremendous effort made to understand the genesis of pain, studies on its pathophysiology is still continuing (Forth *et al*, 1986).

2.2.2. *Pain Perception*

Pain though of varying origins, may be described as acute or chronic. Acute pain most often results from an injury or pathological state and lasts only as long as the tissue lesion itself persists or may be prolonged if treated ineffectively. Other reports suggest that the severity of acute pain influences the development of chronic pain (Forth, 1986, Chapman, 2003).

On the other hand, chronic pain is usually associated with a persistent tissue lesion and may be characterized by sleep disorders, loss of appetite, irritability, loss of interest, loss of contact with surrounding and excessive egocentric preoccupation.

Persistence of severe pain for more than 24 hours induces neuroplasticity associated with the development of intractable chronic pain syndromes (Arnstein, 1997). Neuroplasticity refers to (internal and external) stimuli-induced alteration of neuronal structure and function resulting in new stimulus-response relationships. Chronic pain, acute trauma and psychological trauma are all potent inducers of neuroplasticity.

The manifestation and individual perceptions of pain are determined by the following factors:

i). Four components of pain, namely:

a. Cognitive component, in which the individual becomes aware of the site, duration and intensity of the pain;

b. Affective component, which involves the effect on emotional situation as a result of impairment of well being;

c. Motor component, with movement of facial muscles (expression of pain) rubbing and withdrawal movement (protective function) and;

d. Autonomic component, which the manifestation depends on type of pain, for example in acute pain, there is increased heart rate and blood pressure, deepening of respiration, dilatation of pupils, increased sweat gland secretion and muscle tone.

ii). Psychological influences:

These include an individual's ability to withstand stress. Equivalent experiences will be perceived and reported very differently depending on different individual personality structure and the situation. Psychogenic pain, which may be described, as pain of psychological origin may sometimes be perceived as unbearable, although there may be no discernable tissue lesion.

2.2.3. *Chemical Mediators and Transmitters in the Nociceptive Pathway*

In most cases, stimulation of nociceptive endings in the periphery is chemical in origin. Excessive mechanical or thermal stimuli can cause acute pain, but the persistence of such pain after the stimulus has been removed, or the pain resulting from inflammatory or ischaemic changes in tissues, generally reflects a chemical stimulation of the pain afferents.

The discovery of pain mediators has shown that the generation of pain is closely related to other reactions, namely inflammation and allergy. The main groups of substances that stimulate pain endings in the skin (Rang, 1991), include:

i). Kinins: Among the kinins, the most active substances of pain modulation are bradykinins and kallidin. These are two closely related peptides produced under conditions of tissue injury by the proteolytic cleavage of the active kinins from a precursor protein contained in the plasma (Dray and Perkins, 1993). Bradykinin is a potent pain producing substance, acting partly by release of prostaglandins, which strongly enhance the direct action of bradykinins on the nerve terminals. Bradykinin act by combining with specific receptors of the G-protein coupled type, and produces its cellular effects through production of various intracellular messengers (eg. Phospholipase C and calcium). Specific competitive antagonists have been developed based on the peptide structure of bradykinins. These agents

exhibit analgesic and anti-inflammatory effects and provide a new principle on which future analgesic agents may be based.

ii). Prostaglandins: These agents do not produce pain directly but they strongly enhance the pain producing effect of other agents such as 5-Hydroxytryptamin (5-HT) or bradykinin. Prostaglandins of the E and F-series are usually released in inflammation and also during tissue ischaemia.

Other eicosanoids including prostacyclin, leukotrienes and the unstable hydroxyeicosatetraeinoic acid (HETE) derivatives may also be important.

iii). Metabolites and substances released from active cells excite nociceptive afferent neurons specifically by opening proton-activated cation channels similar or identical to those activated by capsaicin. These agents are mainly of interest as potential mediators of ischaemic pain.

iv). Capsaicin and related irritants: Capsaicin is the active substance in chilli pepper and is responsible for their burning taste. Other spicy plants like ginger and black pepper also contain similar agents. Capsaicin is a highly potent pain-producing substance that selectively stimulates nociceptive and temperature-sensitive nerve endings in tissues, apparently by acting on a specific membrane receptor (James et al, 1993).

v). Neurotransmitters

These include 5-HT and histamine. 5-HT is more active, while histamine is much less active and tends to cause itching rather than actual pain. Both are released locally in inflammation.

Pain that accompanies inflammation and tissue injury probably results from local stimulation of pain fibres and enhanced pain sensitivity (hyperalgia), in part as a consequence of increased excitability of central neurons in the spinal cord (Konttinen et al., 1994).

Bradykinin, released from plasma kininogen, and cytokines such as TNFα, IL-1, and IL-8, appear to be particularly important in eliciting the pain of inflammation. These agents liberate prostaglandins and probably other mediators that promote hyperalgesia. Neuropeptides, such as substance P and Calcitonin gene-related peptides, also may be involved in eliciting pain.

The capacity of prostaglandins to sensitize pain receptors to mechanical and chemical stimulation appears to result from a lowering of the threshold of the polymodal nociceptors of C-fibres.

2.2.4. *Biosynthesis of Prostaglandins*

Eicosanoids are the most universally distributed autacoids in the body. Practically every cell is capable of synthesizing one or more types of prostaglandins or leukotrienes. They are synthesized locally at rates governed by the release of arachidonic acid from membrane lipids in response to appropriate stimuli. The stimuli activate hydrolases, including phospholipase A, probably through increased intracellular calcium ions.

Arachidonic acid is present as a component of phospholipids of cell membranes. Free arachidonic acid is released from tissue phospholipids by the action of phospholipase A_2 via a process controlled by hormones and other stimuli. There are two major pathways in the synthesis of the eicosanoids from arachidonic acid. These are the cyclooxygenase and lipoxygenase pathways.

All tissues have cyclooxygenase and can form cyclic endoperoxides PGG_2 and PGH_2, which are unstable compounds. Further course in a particular tissue depends on the type of isomerase or other enzymes present in it. PGE_2 and $PGF_2\alpha$ are the primary prostaglandins produced.

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Cyclooxygenase (COX) is known to exist in two isoforms COX-1 and COX-2 (Vane et al., 1998). While both isoforms catalyse the same reaction; COX-1 is a constitutive enzyme in most cells. On the other hand, COX-2 is normally present in insignificant amounts but is inducible by cytokines and other stimuli during the inflammatory response (Seibert et al., 1997). It is believed that eicosanoids produced by COX-1 participate in physiological (house keeping) functions such as secretion of mucus for protection of gastric mucosa, haemostasis and maintenance of renal functions, while those produced by

COX-2 lead to inflammatory and other pathological changes. However COX-2 is constitutively expressed in certain areas of the kidney and brain (Breder et al., 1995; Harris et al., 1994)

2.2.5. *Analgesics*

Analgesics are compounds used in pain suppression or agents that cause analgesia – which is the reduction of the awareness of pain and suffering without loss of consciousness. These agents are broadly classified as peripherally acting (or mild) analgesics and centrally acting (or strong) analgesics.

Analgesic drugs fall into the following categories:

1. Non-steroidal anti-inflammatory drugs (NSAID), e. g aspirin and related substance
2. Opioids, like morphine and its congeners and its synthetic compounds.
3. Local anaesthetics.
4. Centrally acting non-opioid drugs e.g antidepressants like amitriptyline
5. Drugs for specific painful conditions e.g carbamazepine, used in trigeminal neuralgia.

Non-Steroidal Anti-inflammatory Drugs (NSAIDs)**:** The non-steroidal anti-inflammatory drugs (NSAIDs) are a group of chemically dissimilar agents used in the treatment of

inflammatory disorders like rheumatoid arthritis, lumbago, etc. (Table 1.2). In addition to their anti-inflammatory effects, they also have analgesic and antipyretic effects.

Most NSAIDs in current use are inhibitors of both COX-1 and COX-2 isoenzymes, though they vary in the degree of inhibition of each (Meade et al, 1993; Mitchell *et al.*, 1993). Selective COX-2 inhibitors like Celecoxib, Rofecoxib and Nimesulide are now available for therapeutic use. Some of these however have been associated with adverse cardiovascular events.

Aspirin, the prototype causes irreversible inactivation of cyclo-oxygenase by acetylating the serine 530 at the apex of the long channel thereby excluding arachidonic acid from the channel (Picto *et al.*, 1994).

Table 2.1 Chemical groups of NSAIDS and their examples.

Chemical class	Examples
Salicylates*	Aspirin, diflunisal, benorylate.
Aryl propionic acids*	Naproxen, ibuprofen, flurbiprofen, fenbufen, ketoprofen.
Indole and indene acetic acids*.	Indomethacin, sulindac.
Fenamates*	Meclofenamic acid, mefenamic acid.
Enolic acids (oxicams)*	Piroxicam, tenoxicam, meloxicam
Pyrozolones*	Phenylbutazone, azapropazone
Heteroaryl acetic acids*	Diclofenac, ketorolac, tolmetin
Diaryl-subtituted furanones**	Rofecoxib
Diaryl-subtituted pyrazolones**	Celecoxib
Indole acetic acids**	Etodolac
Sulfonanilides**	Nimesulide

* = Non-selective COX inhibitors.

** = Selective COX-2 inhibitors.

Not all NSAIDs manifest the three actions earlier specified. Most are analgesics with varying degrees of anti-inflammatory activity. Indomethacin and piroxicam) are strongly anti-inflammatory; naproxen and ibuprofen are moderately anti-inflammatory while paracetamol is devoid of anti-inflammatory activity. Paracetamol effectively inhibits

cyclooxygenases in the brain but not at peripheral tissues, which are sites of inflammation.

General unwanted effects of NSAIDs include;

a. Gastrointestinal disturbance - These include dyspepsia, diarrhea, nausea and vomiting; also gastric damage in chronic users, with risk of haemorrhage, due to inhibition of the protective effect of PGE_2 on gastric mucosa.
b. Skin reactions - This varies from mild rashes, urticaria, and photosensitivity.
c. Renal effects - Reversible renal insufficiency (in individuals who have noradrenergic or angiotensin-mediated vasoconstriction) due to lack of compensatory PGE_2-mediated vasodilation.
d. Analgesic-associated nephropathy; this can occur following chronic use of NSAIDs (more particularly paracetamol).
e. Less commonly liver disorders, bone marrow depression.
f. Haematological effects. Impaired platelet aggregation, because they prevent formation of thromboxane A_2 by the platelets.

Morphine and Morphine like drugs: This class of agents includes various neuropeptides and synthetic analogues whose structures may be quite different from that of morphine. The term 'opioid' is used in reference to any substance that produces morphine-like effect that is blocked by naloxone, a morphine antagonist. Opiates are drugs derived from opium; the dark brown, resinous material obtained from poppy (*Papaver somniferum*)

and includes morphine, codeine, thebaine and a wide variety of semi-synthetic congeners derived from them. Endorphin is generic term referring to the three families of endogenous opioid peptides: the enkephalins, the dynorphins, and β-endorphins. They are however disadvantaged by their relative shorter duration of action (Casy & Parfitt., 1986).

The exogenously administered opiates like morphine have the same receptor sites as their natural opioid peptide analogues.

Opioid Receptors: There is convincing evidence for the three major classes of opioid receptors in the CNS designated mu (μ), delta (δ) and kappa (κ) as well as indications of subtypes within each class (Wood and Iyengar, 1988; Corbett *et al.,* 2003). Receptor binding studies revealed distinct selectivity profiles for each class, while functional studies have established their unique pharmacological profiles buttressing the evidence for the existence of multiple receptors (Martin et al., 1977).

A fourth subtype, б (sigma), was also postulated in order to account for the dysphoric (anxiety, hallucination, bad dreams, etc.) effects produced by some opiates. These are now considered not to be true opiate receptors, since many other types of psychotropic drugs also interact with them (Walker *et al.,* 1990). Of the opioid drugs, only benzomorphans, such as pentazocine and cyclazocine, bind appreciably to б-receptors.

Pharmacological effects associated with opioid receptor subtypes;

Mu (μ) receptor subtype is associated with supraspinal analgesia,respiratory depression, euphoria and physical dependence.

Kappa (κ) receptor subtype is associated with spinal analgesia, miosis and sedation.

Stimulation of sigma (б) receptor subtype produces dysphoria, hallucinations and respiratory vasomotor stimulation.

Delta (δ) receptor subtype exerts effects in limbic structures suggesting an involvement with affective behaviors.

Mu (μ) receptors are preferentially stimulated by morphine. Morphine is a moderate agonist at Kappa and Delta receptors but has little if any agonist action at sigma receptors.

Mechanism of Action of Morphine and Morphine like drugs: Morphine and its related congeners produce their pharmacological effects by acting as agonists and/or antagonists at the various opioid receptors (μ, δ, and κ-receptors). All three receptor subtypes appear to function by primarily causing inhibitory modulation of synaptic transmission in both the CNS and the myenteric plexus on activation. Cloning studies have shown that opioid receptors belong to the family of G-protein-coupled receptors and also act by inhibiting adenylate cyclase, thus reducing the intracellular c-AMP content (West and Millter, 1983; Childers, 1993). Thus morphine and other opioids regulate the transmembrane signaling systems that are characteristically initiated by this kind of receptor (i.e regulation of adenylate cyclase, various ion channels and phospholipids).

All morphine like agonists share morphine's capacity to produce analgesia, respiratory depression, spasm of smooth muscles and physical dependence by stimulating opioid receptors in different locations. They produce decrease in the spontaneous activity of neurons in diverse areas of the central nervous system and in the gastro-intestinal tract, which can be antagonized by naloxone. At least part of the analgesic effect of morphine appears to be due to a selective inhibition of release of neurotransmitter carrying nociceptive stimuli. In addition, in some areas of the brain, like the locus ceruleus, application of opioids reduces spontaneous discharges and responses to noxious stimuli. The hyperpolarizing responses to morphine have been related to an increased potassium conductance. In some instances the increased potassium conductance may be caused by a morphine-induced accumulation of free intracellular calcium. The hyper polarization of neurons appears to be sufficient to decrease either the release of neurotransmitters or the post synaptic responses to excitatory neurotransmitters

Pharmacological Actions of Morphine:

1. Central nervous system;

Morphine has site-specific depressant and stimulant actions in the CNS. The depressant effects include; Analgesia, sedation, mood and subjective effect, depression of respiratory centre, depression of cough center (antitussive activity), depression of the temperature regulating center, etc.

2. Cardiovascular system;

It causes vasodilatation due to; a) Direct action decreasing tone of blood vessels. b) Histamine release. c) Depression of vasomotor center.

3. Gastrointestinal tract;

Morphine causes constipation due to several factors, which include

a) Reduced motility and increased tone of gastrointestinal tract with closing of the sphincters.

b) Decrease in all gastrointestinal secretions.

c) Central action causing inattention to defecation reflex.

4. Other smooth muscles.

a) Biliary tract; Morphine causes spasm of sphincter of Oddi, which may cause biliary colic.
b) Urinary bladder; Tone of both detrusor and sphincter is increased, which leads to urinary urgency and difficulty in micturation.

c) Bronchi; Morphine causes histamine release which can cause bronchoconstriction.

2.3 The Flavonoids

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. To date about 3000 varieties of flavonoids are known (Kuhnau, 1976). Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity (Cesarone *et al*., 1992). Other biological effects exhibited by flavonoids include anti-inflammatory, anti-hepatotoxic and anti-ulcer actions (Robak *et al*., 1988). They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are potent antioxidants and have free radical scavenging abilities. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality (Hertog *et al*., 1993). They have been shown to inhibit the growth of various cancer cell lines in vitro, and reduce tumour development in experimental animals (Mori *et al*., 1988).

Flavonoids are divided into subclasses,as follows;

a) Flavonols; these include morin, rutin, quercetin, quercetrin, galangin, robinin, fisetin.
b) Flavonones; these include hesperidin, naringin, naringenin, hesperitin, lukvertin, pinocembrin.
c) Flavones; these include apigenin, tangeretin, flavone, baokalein, luteolin, chrysin.

d) Flavonolols; these include silibinin, silymarin, taxifolin, pinobanksin.

e) Flavan-3-ols; an example is catechin.

f) Isoflavones; examples include genistein and daidzin.

Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavones and flavonols are collectively the most abundant group of compounds present in the plant kingdom.

2.3.1 *Pharmacological Effects of Flavonoids*

I. Central nervous system (CNS) Activity: Synthetic flavonoid like 6-bromoflavone was shown to displace flumazenil binding to membranes from rat cerebellum but not from spinal cord, indicating selectivity for the B_2-omega receptor subtype. Similar results in rats showed that some synthetic flavonoids possess anxiolytic like properties similar or superior to that of diazepam (Griebel et al., 1999).

II. Cardiovascular system:

Flavonoids have been reported to have action on the heart. The flavone β_2-exerts coronary dilatory activity and was commercially available under the name 'chromocor' and its combination with rutin and isoquercetin was also available with brand name 'flavoce',useful in the treatment of artherosclerosis.(Sanchez *et al.*,1996). 3-methyl quercetin has positive chronotropic effect on guinea pig right atrium and antiarrythmic effect on left atrium. (Lackeman *et al.*, 1986).

In recent report, the cardiotoxicity (negative inotropic effect) of doxorubicin on the mouse left atrium has been inhibited by flavonoids (Huesken *et al*., 1995).

III. Gastrointestinal tract:

a) Antiulcer activity:

Oral treatment with the ether fraction of the flavonoids extract demonstrated a good level of gastric protection. Mucous content was increased and accompanied by proportionate increase in proteins and hexosamines (Alarcon *et al.*, 1994).

Quercetin and rutin were found to produce an inhibitory effect on intestinal functions, and that their actions are mediated through α_2-adrenergic and calcium systems (Carlo *et al.*, 1994). This result may show the beneficial effects in diarrhea and other intestinal secretions. Lorenz et al showed that (+) cyanidanol-3 has histidine decarboxylase inhibitory activity and hence anti ulcer activity (Lorenz *et al*., 1973).

b) Hepato-protective activity:

In a study carried out to investigate silymarin, apeginin, qurecetin and naringenin as putative therapeutic agents against microcrystin LR-induced hepatotoxicity, silymarin was found to be the most effective one (Carlo *et al*., 1993). Rutin and venorutin showed regenerative and hepato-protective effects in experimental cirrhosis (Lorenz *et al*., 1973).

IV Antioxidant activity:

The increasing acceptance of free radicals as common and important biochemical intermediates has been implicated in a large number of human diseases (Wegener *et a*l., 1999).

Quercetin, morin and rutin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, anti-allergic, antiviral as well as anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases.

Quercetin and silybin acting as free radical scavengers were shown to exert a protective effect in reperfusion ischaemic tissue damage (Hillwel, 1994)

V Anti-inflammatory activity:

A number of flavonoids are reported to possess anti-inflammatory activity. Hesperidin, a citrus flavonoid possesses significant anti-inflammatory and analgesic effects (Shahidi *et al.*, 1998). Recently, apigenin, quercetin have been reported to exhibit anti-inflammatory activity. Treatment with silymarin-demonstrated reversal of the carrageenin induced biochemical changes. Qurcetin,gallic acid ethyl ester and some other flavonoids might account for the antinociceptive action reported for the hydroalcoholic extract of *Phyllanthus carolininsis* (Alcarraz *et al*, 1987).

VI. Anti-neoplastic activity

Detailed studies have revealed that quercetin exerted a dose - dependent inhibition of cell growth and colony formation. The flavonoids catechin, toxifolin and fisetin also suppressed cell growth (Hirano *et al.,* 1993).

VII. Effects on blood vessels.

Flavonoids,like tangeratin, hesperidin,quercetin and rutin have been found to reduce aggregation of horse erythrocytes. The decrease in blood aggregation produced by most of the flavonoids may explain the reported beneficial effects of these compounds on abnormal capillary permeability and fragility, the reduction of disease symptoms and their protection against various traumas and stresses (Wild *et al*., 1969).

Quercetin and rutin have been used as effective constituents of several pharmaceuticals employed in the treatment of capillary fragility and phlebosclerosis. Other flavonoids like rutin, hesperitin etc were found to inhibit capillary permeability and Arthus Phenomenon (Wenner *et al*., 1996). Orally administered flavonoids weakly inhibit the vascular permeability and prevent pulmonary haemorrhage. Acacetin given at 25-100mg/kg oral doses to mice reduced capillary fragility and at 50-100mg/kg reduced vascular permeability (Gerdin *et al.*, 1983).

VIII. Anti-microbial activity:

Flavonoids and esters of phenolic acids were investigated for antibacterial, antifungal and antiviral activities. All samples were active against the fungal and gram-positive bacterial test strains and most showed antiviral activity (Kujumgier *et al.,* 1999).

2.3.2 *Biochemical effects of Flavonoids*

.i) On enzymes:

Flavonoids are known to inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{2+}-ATPase, lipooxygenase and cyclooxygenase. (Baumann et al., 1980, Koch *et al.*, 1992).

Quercetin and morin are effective in antagonizing bradykinin responses (Calixto *et al*., 1991).

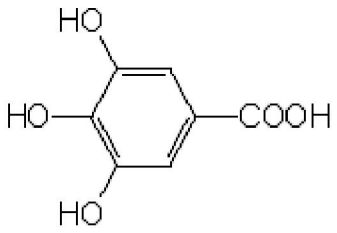
Flavonoids also inhibit intracellular Ca^{2+} elevation by reducing phospholipase-C activity (Kyo *et al*., 1998).

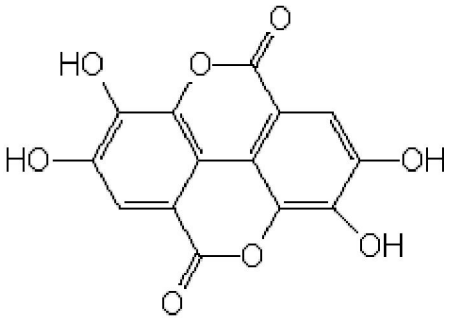
ii) On hormones

Flavonoids have also been shown to have regulatory activity on hormones, by binding to 17 beta-hydroxy steroid dehydrogenases, which regulates estrogen and androgen levels and to 3 beta-hydroxy steroid dehydrogenase, which regulates progestin and androgen levels in humans (Beladi *et al*., 1987).

2.4**.** **Tannins.**

Tannins comprise a large group of natural products widely distributed in the plant kingdom. They have a great structural diversity, but are usually divided into two basic groups: the hydrolyzable type and the condensed type. Hydrolyzable tannins include the commonly occurring gallic acid and the ellagic acid.



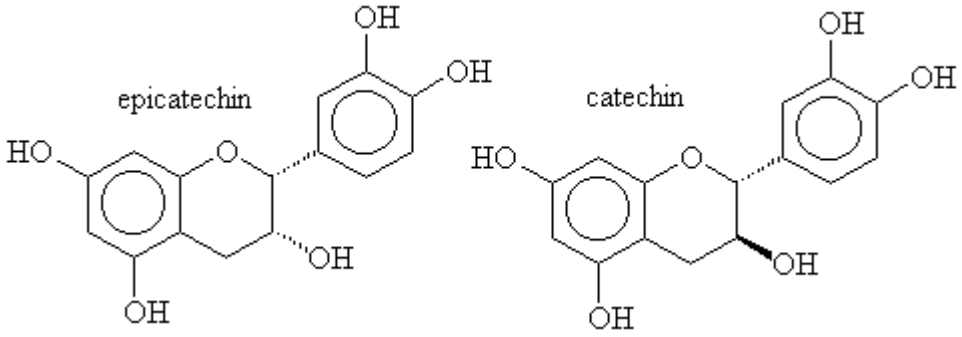


Gallic acid Ellagic acid

Hydrolyzable tannins are readily degraded into smaller molecules and are found in many plant species but particularly in high concentrations in nutgalls growing on *Rhus semialata* (Chinese and Korean gallotannins) and *Quercus infectoria* (Turkish and Chinese gallotannins), the seedpods of *Caesalpinia spinosa* (Tara tannins), and the fruits of *Terminalia chebula*. They react with proteins to produce the typical tanning effect of leather. Medicinally, this is important for treatment of inflamed or ulcerated tissues. They also contribute most of the astringent property that is noted when drinking tannin-containing beverages.

The condensed tannins, also known as proanthocyanidins, are much more resistant to decomposition and merely yield polymers or precipitates when acidified. The basic monomers of condensed tannins are epicatechin and catechin (identical except for orientation of the molecules). These are then extended by the successive addition of similar phenol units to produce polymers (polyphenols). Traditionally, important commercial sources of condensed tannins are the heartwood of *Schinopsis spp*. (quebracho tannins), the bark and/or heartwood of *Acacia catechu* (catechu tannins) and *Acacia mollisima* (mimosa tannins), and the bark of *Rhizophora* (mangrove) and *Eucalyptus* species. The application of proanthocyanidins as health protective

antioxidants was popularized through extracts of pine bark (particularly the branded product Pycnogenol) and grape seeds, which can be used interchangeably. In addition, proanthocyanidins were recognized as beneficial for vision, and are active components of bilberry extract sold for this purpose.(Subhuti.,2003).



Although both types of tannin have been used to treat diseases in traditional medicine, the hydrolyzable tannins have long been considered official medicinal agents in Europe and North America. They have been included in many pharmacopoeias. In the older editions in particular, they are specifically referred to as tannic acid and were recommended for the treatment of inflammation and ulceration, including topical application for skin diseases and internally for intestinal ulceration and diarrhea. The condensed tannins also have important medicinal roles as stable and potent antioxidants. In China, tannin-containing herbs are listed in the Materia Medica and are used in treating:

- Intestinal disorders, such as diarrhea and dysentery, intestinal parasites, rectal prolapse, hemorrhoids;

- Bleeding, including functional bleeding, hematochezia (blood in the stool), bleeding hemorrhoids, and topically for bleeding wounds and ulcerations; and
- Excessive discharge, such as enuresis and frequent urination; leucorrhea; hyperhidrosis (excessive sweating) and night sweating; involuntary seminal emission.

Table 2.2. some herbs with tannins as major component and their uses.

Herb (Botanical Name/Pinyin)	**Taste, Nature**	**Applications**
Acacia catechu gall (*ercha*) [catechu gall]	bitter, astringent, neutral	cough, red and white dysentery; topically for skin ulceration
Cedrela sinensis root bark (*chungenbaipi*) [Chinese cedar]	bitter, astringent, cool	red-white dysentery, hematochezia, morbid leucorrhea, functional bleeding, involuntary emission
Punica granatum rind (*shiliupi*) [pomegranate]	astringent, warm	chronic diarrhea and dysentery, hematochezia, rectal prolapse, involuntary emission, functional bleeding, morbid leucorrhea, intestinal parasites

2.5. **Amino Acids and Proteins**.

In plants, amino acids are broken down into two groups, protein, and non-protein. There are twenty amino acids, derived from the acid hydrolysates of plant proteins (as with animal proteins). Plant proteins are essential for carrying out specific cellular functions both internally and externally. Plant proteins are seed-based store-houses

for nitrogen and guard against would-be predators. Some are toxic to humans but some are of daily necessity in the human diet. Some of them have been developed into specific drugs like: L-Dopa, from fava beans used in the treatment of Parkinson's disease; L-Cysteine, used in eye drops and topical antibiotics. L-Arganine stimulates the pituitary gland to release growth hormone while L-Aspartic acid present in coffee, liquorice, sugar cane and sugar beet is neuro-excitatory.

2.6**. Carbohydrates and Related compounds**

Plant energy storage components are referred to as carbohydrates. Carbohydrates include mono- (sucrose, lactose, etc.), and poly- (starch, inulin) saccharides, some acids which are produced after cellular carbohydrate respiration, alcohols such as sorbitol and cellulose; and gums and mucilages. For the purposes of therapeutics, the polysaccharide and gum/mucilage subgroups are most important.

Polysaccharides are known to exert a beneficial action on the body's immune system. They are produced through the linkage of simple or single sugars linked by ethers in various and complex ways, and are divided into two categories, water-soluble or water non-soluble. Plant starch, gums, mucilage, cellulose (and sub-group hemicellulose) are all polysaccharides.

Cellulose, i.e., cotton, powdered cellulose, microcrystalline cellulose, and purified rayon are used as bulking agents for alleviating constipation, as opthalmic solutions, topical emollients and protectants, and as appetite suppressant.

It has been difficult for phytochemists to distinguish between gums and mucilage

categorically. Presently, it is generally agreed that while gums are water solvent, mucilage will become a slimy mass; and, that gums are pathologically formed while mucilages are physiological in origin.

Mucilage, therapeutically, can reduce bowel irritation, gut irritation, peristalsis, toxin absorption, cough, bronchial and urinary spasm. Mucilage can also increase expectoration. As a gelatin agent, the polysaccharide hydrocolloidal carrageenan often finds its way in ice cream; it is also used as a laxative ingredient.

2.7 Steroidal Compounds

Steroids develop and control the reproductive tract in humans, molt insects, induce sexual reproduction in aquatic fungi. Therapeutically, steroids are cardiotonic (digitoxin), Vitamin D precursors, oral contraceptives (semi-synthetic progestins), anti inflammatory agents (corticosteroids) and anabolic agents (androgens). Structurally, steroidal compound always includes a 4-membered hydrocarbon ring.

In plants, steroidal content is divided into saponins and alkaloids. In humans, steroidal compounds are used as topical antibiotics and in relieving dysmenorrhea (Hellen, 1995).

2.8. Objectives

The plant *Pterocarpus erinaceus* has been reported to be ethno medically used in fever, diarrhea, dysentery and as an ingredient in abortifacient preparations (Dalziel, 1948). Hamisu Sangarib (a herbalist) also uses it in the control of bleeding in Kano, Nigeria.

This research work therefore intends to scientifically verify these claims using the following specific objectives of study.

i. Extraction of stem bark of the plant using 70% ethanol.

ii. Phytochemical screening of the crude ethanolic extract.

iii. The analgesic activity of the stem bark extract using the following models of pain.

. a. Centrally acting pain models like the hot water tail immersion test in rats.

b. Peripherally acting pain models like the acetic acid-induced writhing in mice

iv. Anti-inflammatory activity of the extract using the egg albumin - induced hind paw oedema test in rats

v. Effect of the extract on isolated smooth muscle preparations of pregnant rat uterus, rabbit jejunum and guinea pig ileum

vi. Effect of the extract on blood cells and haemostasis; these include evaluating haemoglobin concentration, white blood cells and their differentials and platelet counts. Bleeding and clotting times will also be studied.

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vii. Acute toxicity study of the extract on rats using the Lorke' s method after oral and intraperitoneal administration.

Chapter 3

MATERIALS AND METHODS

3.1 Laboratory Equipment.

The equipment used in the studies include; Weighing balances (Mettler Toledo model AB 54 maximum of 50g) and master model (1g-4000g),stop clock, syringe and needle (1ml,2ml,5ml),centrifuge machine (centrum 4040 series), microscope, Ugo basile microdynamometer 7050, organ bath (25ml), hand gloves, measuring cylinders, conical flasks, mortal & pestle (ceramic),dissecting kit, beakers, water bath, digital plethysmometer, sterile lancet .

3.2 Drugs and chemicals

Drugs and chemicals used include; Histamine (Sigma,94H0727), Oxytocin (Sigma,83H58052), Acetylcholine (Sigma,35H0784), 99% ethanol, 0.6% acetic acid, pentazocine(Rambaxy), piroxicam, aspirin, Molisch's reagent, Dragendorff's reagent, Wagner's reagent, heparin.

3.3 Laboratory Animals.

Mature wistar rats (160-250g) and mice (40-60g) of both sexes were purchased from the National Veterinary Research Institute Vom, Nigeria and Faculty of Veterinary Medicine, Ahmadu Bello University Zaria Nigeria, respectively. They were kept in clean cages

under 12/12 hours normal light/dark cycle and allowed to acclimatize to the laboratory environment for a period of four (4) weeks before the commencement of the experiment. They were fed on 24% protein, Pfizer products Lagos, Nigeria and water *ad libitum* during the stabilization period. They were identified by marks on their tails and by cage number.

Guinea pigs

Guinea pigs (300 – 350g) of both sexes used in the experiment were purchased locally from Samaru market, Zaria, Nigeria. They were kept in animal house and allowed to adjust to laboratory environment for a period of three (3) weeks before commencement of the experiment. They were fed with carrot leaves and provided water *ad libitum* during the stabilization period.

3.4 Collection of Plant Material

The stem bark of *Pterocarpus erinaceus* was collected along Burunburun village in Tudunwada local government area of Kano state, Nigeria in November 2004. The plant was taxonomically identified and authenticated by Mal. Musa of the Botany Department of Ahmadu Bello University Zaria, Nigeria.

A voucher specimen (No 900063) was made and deposited at the herbarium.

3.5 Extract Preparation.

The stem bark was air-dried for 30 days. It was then reduced to powdered form by grinding in pestle and motar.

One hundred and sixty grams (160g) of the powdered stem bark was cold macerated in 1000ml of 70% ethanol for 24 hours with constant shaking and filtered using Whatmans filter paper No.1.It was then concentrated to dryness on a water bath. The percentage yield was calculated and the crude extract was kept in a dessicator. When required, a known quantity of the extract was taken, dissolved in a known volume of distilled water to obtain the desired concentration.

3.6. **Phytochemical Studies**.

Phytochemical analyses of the extract were performed according to the methods of Odebiyi and Sofowora (1978) and Trease and Evans (1983). The extract was screened for the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, proteins and steroids.

3.6.1 *Test for Carbohydrates*

The general test used was the Molisch's test. Three drops of Molisch's reagent was added to 2 ml of aqueous solution of *Pterocarpus erinaceus* powder. Concentrated sulphuric acid was then allowed to run down the inclined tube slowly. The interphase was observed for a purple colour, which is characteristic of carbohydrates. Control test was carried out by similar procedure using distilled water in place of aqueous solution of stem bark as reference.

3.6.2 *Test for Reducing and Non-reducing Sugar*

i. One gram (1g) of *Pterocarpus erinaceus* powder was dissolved in 10 ml of distilled water, heated for about 2 minutes and 1 ml Fehling's solution was added to it. A brick red colour reaction is indicative of the presence of reducing sugar.

ii. One gram (1g) of *Pterocarpus erinaceus* powder was dissolved in 10 ml distilled water and heated for about 2 minutes. 10 ml of dilute H_2SO_4 was added followed by 1 ml Fehling's solution. A red precipitate is indicative of non-reducing sugars.

3.6.3. *Test for Tannins*

Two grams (2g) of stem bark powder of *Pterocarpus erinaceus* was boiled in distilled water for 5minuites. It was filtered and made up to 50ml volume.1ml of the solution was added to 5 ml of distilled water followed by 0.5 g acid sodium phosphate and heated for 1min.It was filtered and the filtrate treated with 1ml 2% phenazone. Milky/cloudy precipitates give a positive reaction.

3.6.4. *Test for Saponins*

One hundred milligrams (100 mg) of the powdered stem bark was added into 5 ml of distilled water and heated for 1 min. It was centrifuged, the supernatant diluted with 1 ml of distilled water and shaken to observe frothing which indicates the presence of saponins.

3.6.5**.** *Test for Terpenes and Steroids*

Two grams (2 g) of powdered stem bark was boiled with 5 ml of 95% ethyl alcohol in a steam bath, filtered and the filtrate evaporated to dryness. The residue was then dissolved

in 10 ml of anhydrous chloroform and filtered. This was divided into 2 portions and used for the following tests.

Lieberman-Burchard Test:

The first portion was mixed with 1 ml of acetic anhydride; the tube inclined and 1 ml of concentrated sulphuric acid was added. The test tube was observed for green and red colours at the lower layer for steroids and triterpenes respectively.

Salkowski's Test:

The second portion was mixed with 2 ml of concentrated H_2SO_4 carefully to form two layers with an interface of a reddish brown colour indicative of steroid ring.

3.6.6. *Test for Flavonoids*

One gram (1g) of extract was added with 2ml acetone and warmed. The residue was mixed with 10ml-distilled water and heated. It was filtered hot and cooled.10 percent lead acetate solution was added to 2ml of the filtrate and observed for yellow colored precipitate.

3.6.7. *Test for Alkaloids*

Three grams (3.0g) of the crude ethanolic extract was dissolved in 5ml methanol. The solution was evaporated to dryness and 500mg of the residue was mixed with 10ml of 1% aqueous hydrochloric acid on a water bath. A One ml portion of the filtrate was each

treated with three drops of the following reagents: Dragendorff's reagent (potassium bismuth iodine solution) and Wagner's reagent (solution of iodine in potassium iodide). Deep brown coloured precipitate and turbidity with Dragendorff's and Wagner's reagents are indicative of presence of alkaloids in the extract (Sofowora, 1982).

3.6.8. *Test for Proteins and Free Amino Acids*

Two grams (2gm) of the ethanolic extract was dissolved in 2ml of water to which three drops of Millions reagent was added. A white precipitate that turns red on heating is indicative of presence of proteins.

3.7 Acute Toxicity Studies

Acute toxicity study was conducted using modified Lorke's method (1983). Thirteen (13) rats of both sexes were used. The evaluation was done in two phases. In phase one, three groups of three rats each, were treated with 10, 100 and 1000 mg/kg of extract intraperitoneal (*i.p*) respectively. The rats were observed for clinical signs of toxicity and death for 72 hours.

Based on the results of phase1, 4 fresh rats were each treated with 300, 400, 500 and 600 mg/kg extract (*i.p*) respectively in the second phase. The LD_{50} was then calculated as the geometric mean of the highest non-lethal and lowest lethal dose.

The toxicity test by oral route was also investigated using the same method. In the second phase, doses of 1500mg, 3000mg and 5000mg/kg extract were administered and the rats were observed for clinical signs of toxicity and death for 72hrs.

3.8 **Sub-acute Toxicity studies**

This study was carried out for a period of 30 days.

Twenty (20) adult male rats were divided into four groups of five (5) rats per group. The first served as the control and was given 0.2ml normal saline. The remaining three groups were given 50, 100 and 200mg extract per kilogram body weight orally respectively via a cannula daily. The rats were fed regularly and given water *ad libitum.*

3.8.1 *Bleeding Time Determination***.**

At the end of the thirty days, the base of each rat's tail was cleansed with a swab and pricked with a sterile lancet and a stop clock was started immediately. Blood was blotted every 15 seconds using Whatman filter paper until bleeding ceased. Time taken for the blood to stop dripping is the bleeding time.

3.8.2 *Clotting Time Determination***.**

A cut on the distal part of each rat's tail using a sterile scissors was made and blood was placed on a grease-free glass slide and a stop clock was started immediately. A needle was passed through the blood on the glass slide every 15 seconds until a thread-like structure was seen. The time taken for the thread-like structure to form was taken as the clotting time.

Blood samples were collected for estimation of haematological parameters (packed cell volume (PCV), haemoglobin concentration (Hb), platelet count, white blood cell (WBC)

count and differential WBC count) into EDTA (anticoagulant) containing bottles. Estimations were done using the methods of Tietz (1985).

3.8.3 *Packed Cell Volume (PCV) Determination*

The EDTA blood sample was placed in a tube and the dry end of the tube was rapidly sealed by heating in a fire flame. This was then centrifuged using micro-haematocrit centrifuge for 5 minutes, after which the denser red cells sank to the bottom. The slightly less dense white cells formed an intermediate layer and the top layer was the plasma. The packed cell volume was then read off directly in the micro-haematocrit reader.

3.8.4. *Haemoglobin (Hb) Determination*

A drop of blood was placed on the side of the glass wedge of the haemoglobinometer. The red blood cells were haemolysed. The glass wedge was then fixed into haemoglobinometer, its eye piece focused while the scale was gradually moved to get the haemoglobin value.

3.8.5. *White Blood Cell (WBC) Count Determination*

The EDTA blood sample was placed into the white cell pipette up to the 0.5ml mark and 0.02ml of the blood was poured into a tube containing 0.35ml of WBC diluting fluid (1g of glacial acetic acid dissolved in 100ml of water and a drop of gentian violet). This gave a dilution of 1:20. After gentle mixing, it was allowed to stand for 5-10 minutes. The

diluted blood was then introduced into the counting chamber by approximating the tip of the pipette to one end of the chamber (the cover slip being previously in position). The drop of blood was moved up the chamber by capillary action and allowed to settle down. The chamber was then focused at X 10 eyepiece of the microscope to get the counting under the focus. White blood cells in four squares of the counting chamber were counted. The white cells appeared as spherical bodies scattered about the square.

WBC calculation:

Suppose the number of cells is N

The volume of the chamber is $4/100mm^3 = 0.4mm^3$

Dilution is 1:20.

Then $1mm^3$ of undiluted blood contains N x 20/0.4 i.e N x 50 cells/mm^3

3.8.6. *Differential Count (DC) of White Blood Cell Determination*

A drop of the EDTA blood sample was placed at the end of a slide. The grounded edge of another slide was placed at an acute angle in front of and on top of the slide containing the drop of blood, until the blood ran along the edge of the slide. The spreader slide was then pushed forward so that a fine film blood was obtained. The film was dried quickly in air and stained by pouring sufficient Leishman's stain to cover the dry film and it was allowed to stand for about 2 minutes. The stain on the slide was then mixed with buffered distilled water and left for about 10 minutes. The fluid was then poured off and the film covered with buffered distilled water to wash the slide. The slide was dried, after which a drop of oil immersion was placed on the dried stained film and examined under the microscope at X100 magnification. The microscope was given maximum illumination to

ensure a clear view of cells. White blood cells also known as leucocytes are nucleated cells and some of them are capable of amoebic movement.

Leucocytes consist of monocytes, neutrophils, lymphocytes, eosinophils and basophil which can be distinguished according to their staining reactions and the colours produced in them. Monocytes when stained are pale violet; neutrophils usually stain a light pink colour in the cytoplasm and purple violet in the lobe nucleus; lymphocytes show 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 kidney shaped nuclei and their cytoplasm contain masses of large granules which stain deep purple frequently obscuring the nuclei.

3.8.7 *Determination of Platelet Counts*

The method of Dacie and Lewis (1984) was used. For platelet count, blood was taken from each rat by tail-end amputation and sucked up to the 0.01 ml mark of white blood cell pipette. This was introduced into 0.19ml of Boar's fluid in a test tube and mixed thoroughly. The mixture was gently dropped onto the counting chamber, covered with a cover slip, and using 'x 40' objective lens, platelets were counted.

3.9 Analgesic Activity

3.9.1 *Acetic Acid-Induced Writhings in Mice*

The method described by Koster et al (1959) was used. Twenty Swiss albino mice were divided into four groups of 5 mice per group. The first group served as control and received 0.2ml normal saline, the second and third groups received 50 and 100mg/kg of the extract respectively, while the fourth group was given150mg/kg acetyl salicylic acid (reference drug) intraperitoneally. After 30 minutes, (10ml/kg) 0.6% v/v acetic acid solution in distilled water was administered to all mice in the groups. Each mouse was then placed in a transparent Perspex observation box and the number of writhes that occurred between 5 and 15 minutes after acetic acid were then counted.

Percentage inhibition of writhes was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (control) x mean No. of writhes (test)}}{\text{Mean No. of writhes (control)}} \times 100$$

3.9.2. *Hot Water Tail Immersion Test*

Rats used for this study were screened for sensitivity test by immersing tip of the tail gently into hot water ($55^{o}C - 55.5^{o}C$) and rats that lifted the tail from hot water within 5 sec. were selected for the study, after which thirty (30) rats (6 rats per group), were then selected. The first group served as control and received 0.2ml normal saline, while the rats in the second, third and fourth groups received the extract at doses of 50,100 and 200mg/kg respectively. Rats in the fifth group were given pentazocine 5mg/kg (reference

drug) intraperitoneally. The reaction time (time taken for rat to remove tail from water) was measured at 0, 15, 30, 45, 60, 75 and 90 minutes respectively (Sewell *et al.*, 1974).

3.10 Antiinflammatory Studies

The method of Winter *et al.* (1962) as slightly modified by Akah and Nnambie (1994) was used. Five groups of five rats were pretreated as follows: The control group was given 0.2ml normal saline; the second group was given piroxicam (5mg/kg) intraperitoneally. The remaining three groups received the extract at doses of 50, 100, and 200mg/kg respectively. After 30 minutes the rat in each group was injected with 0.1ml of fresh raw egg albumin subcutaneously to the plantar surface of the right hind paw. The measurement of the paw oedema was carried out by a displacement technique using Digital Plethysmometer (LE 7150) before and every 20 minutes after the injection of egg albumin for 2 hrs.

3.11 Test on Isolated Pregnant Rat Uterus

Female pregnant Wistar rat weighing 180g was killed by a blow on the head, exsanguinated and the abdomen exposed, foetus were removed. The horns of the uterus were freed and dissected out of the adhering tissue. A piece measuring about 2 cm was cut out and mounted in a 25ml organ bath with Dejalon's solution.

The solution was maintained at 37 $\pm$ 1^{o}C, aerated with 95% oxygen and 5% carbon dioxide. A 60 min equilibration period was allowed during which the physiological solution was changed very 15 min. At the end of the equilibration period, the effects of oxytocin (0.002-0.008iu/ml) and the extract (4ug/ml-6.4mg/ml) were investigated.

The contact time for each concentration was 60 sec, which was followed by washing three times. The tissue was allowed to rest for 15 min before the next addition. Responses were isometrically recorded with a micro dynamometer Ugo Basile Unirecorder 7050.

3.11.1 *Preparation of Dejalon's Solution*

90g Nacl, 15g D-glucose, 5g $NaHCO_3$, were weighed and placed in a clean container. 500mls of deionized water was added to dissolve the salts and stirred continuously. 42ml KCl 10%, and 2.7ml $CaCl_2$ (molar) were added respectively with continous stirring. Deionized water was then added to make up to 10 L volume.

3.12 **Studies on the Isolated Rabbit Jejunum**

The rabbit was starved for one day before use, killed by a blow on the head, exsanguinated and the abdomen was opened. Segments of the jejunum about 2-3cm long were removed and dissected free of adhering mesentery. The intestinal content was removed by flushing with Tyrode solution.

The tissue was mounted in a 25ml organ bath containing Tyrode's solution at $37^{o} \pm 1^{o}C$ and aerated with air. A 60 min equilibration period was allowed during which the physiological solutions was changed every 15 min. At the end of the equilibration period, the effects acetylcholine and extractl were investigated. The contact time for each concentration was 60 sec, which was followed by washing three times. The tissue was allowed resting period of 15 min before the next addition. Responses were recorded by a recording micro dynamometer. (Amos *et al*., 1998).

3.12.1. *Preparation of Tyrode Solution*

90g Nacl, 10g D-glucose, 10g $NaHCO_3$, were weighed and placed in a clean container. 500mls of deionized water was added to dissolve the salts and stirred continuously. 5ml NaH_2PO4 $2H_2O$ 10%, 1ml $MgCl_2$ $6H_2O$, 20ml $CaCl_2$ and 20ml KCl 10% were added respectively with continous stirring. Deionized water was then added to make up to 10L volume.

3.13 **Studies on Guinea pig Ileum**

Guinea pigs of either sex were starved of feed over night, killed by a blow on the head, exsanguinated and the abdomen opened. A segment of the ileum (2-3cm) was taken and dissected free of adhering mesentery and mounted in a 20ml organ bath containing

Tyrode solution aerated with air and maintained at 37^{o}C.The tissue was equilibrated for 60min with the Tyrode solution replaced after every 10min.At the end of the equilibration period, effects of the extract (8µg/ml-3.2mg/ml), Acetylcholine (0.04-0.16µg/ml) and histamine (0.8-3.2µg/ml) were investigated on the preparation and recorded on Ugo Basile Unirecorder 7050.

3.14. Statistical Analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). The data was statistically analyzed using student's t-test and differences between means were considered significant when $P<0.05$.

CHAPTER 4 RESULTS

4.1 Preliminary Phytochemical Screening of Stem-bark Extract of *Pterocarpus erinaceus*

The phytochemical analyses of the extract of *P.erinaceus* gave positive reactions to tannins, flavonoids, steroids, carbohydrates, proteins and amino acids. The extract however gave negative reactions to alkaloids and saponins (Table 4.1).

Table 4.1 Phytochemical Constituents of the Ethanolic Stem - Bark Extract of *Pterocarpus erinaceus.*

Components	Remarks
Flavonoids	++
Carbohydrates	++
Proteins and Amino Acids	++
Tannins	++
Steroids	++
Saponins	-
Alkaloids	-

++ Positive (available)
- Negative (not available)

4.2 Acute Toxicity Test. (LD_{50})

In the first phase of the experiment all the rats in the group treated with 1000mg/kg extract died. In the second phase, rats treated with 300 and 400mg/kg extract did not die while mortality was observed in rats treated with 500 and 600mg/kg, intraperitoneally. The calculated median lethal dose (LD_{50}) for ethanolic stem bark extract of *Pterocarpus erinaceus* intraperitoneally was 447.21mg/kg (Table4.2). However, none of the rats treated orally died, that is the LD_{50} was found to be greater than 5000mg/kg.

Table 4.2 Acute Toxicity Studies of Ethanolic Extract of the Bark of *P. erinaceus* in Rats.

Dose mg/kg,*i.p*	No. of death/used	Mortality %
300	0/1	0
400	0/1	0
500	1/1	100
600	1/1	100

LD_{50} = Geometric mean of 400mg x 500mg =$\sqrt{400 \times 500}$ = 447.21mg/kg, *i.p.*

4.3 Sub-Acute Toxicity Studies (Haematological Indices)

The bleeding and clotting times obtained for rats treated with the *P. erinaceus* 50 and 100mg extract per kilogram dose groups were not significantly different from the control, while significant ($P<0.05$) decrease in bleeding time was observed in rats that received 200mg extract per kilogram body weight (Table 4.3). The clotting time slightly decreased in rats treated with the 200mg/kg dose (Table 4.4).

The platelet count was insignificantly reduced at 50 and 200mg/kg and increased at 100mg/kg doses. Haematological parameters like haemoglobin concentration, packed cell volume, white blood cell and differentials and platelet count were also not significantly affected by all doses of *P. erinaceus* extract used (Table 4.5).

Table 4.3. Effect of *P. erinaceus* on the Bleeding Time of Rats**.**

Treatment P.O	Mean bleeding time (sec)
N/saline	17.75 $\pm$ 2.25
Extract 50mg/kg	17.25 $\pm$ 1.70
Extract 100mg/kg	16.75 $\pm$ 1.90
Extract 200mg/kg	11.5 $\pm$ 1.20*

n = 5 * significantly different from control at $P < 0.05$

Table 4.4 Effect of *P. erinaceus* on the Clotting Time of Rats.

Time (sec)

Treatment P.O.	Mean clotting time (Sec)
N/Saline	45.5 ± 4.09
Extract 50mg/kg	45.5± 2.39
Extract 100mg/kg	45.75±7.00
Extract 200mg/kg	42.75±3.17

Table 4.5 Effect of Administration (30 days) of *P. erinaceus* Stem-bark Extract (50-200mg/kg, *P.O*) on Some Haematological Parameters in Rats**.**

Treatment *P.O.*	Hb count (g/dl)	PCV (%)	W.B.C. count ($x10^7$/L)	Neutrophils (%)	Lymphocytes (%)	Platelets x 10^9/L
N/Saline	13.1±.23	45.3±.45	12.3±1.3	26.0±3.5	67.2 ±6.19	733.6 ± 61.0
P.e 50mg/kg	13.9±.28	47.5±.65	11.9±2.4	21.0±2.2	81.0 ± 2.62	711.4 ± 37.0
P.e 100mg	14.2± .16	48.8± 1.5	12.6±1.9	31.0±3.2	66.6 ± 3.47	762.8 ± 48.1
P.e 200mg	13.8±.45	46.3± 1.9	13.6±2.6	25.0±1.8	72.4 ± 2.94	677.0 ± 90.0

4.4 Analgesic Studies

4.4.1 *Acetic acid-induced Writhing in Mice.*

The extract significantly ($P<0.01$) decreased the number of acetic acid-induced abdominal constrictions in mice dose dependently at 50 and 100mg/kg when compared to control.

Aspirin (150mg/kg) also produced significant inhibition of the abdominal constriction (33.7%) which was lower than that produced by the higher dose (100mg/kg) of the extract (61.3%) used (Table 4.6).

4.4.2 *Tail Immersion Studies*

The extract produced a dose - related significant prolongation of the reaction time ($P<0.05$-0.001) to thermally - induced pain produced on hot tail immersion compared with the control. Pentazocine (5mg/ml) elicited a higher increase in reaction time than the extract.

The increase in reaction time was time- dependent for both extract and pentazocine at 45 minutes (Table 4.7).

Table 4.6 Effect of Ethanolic Stem-bark Extract of *P. erinaceus* on Acetic acid-induced Abdominal Contractions in Mice.

Group	Dose mg/kg, ip	Mean no. of writhing ± SEM	% Inhibition of writhing
Control	Saline	26.4 ± 3.6	-
P. erinaceus	50	18.8 ± 3.7	28.9
P. erinaceus	100	10.2 ± 3.2*	61.3
ASA	150	17.5 ± 4.5	33.7

n=5 * P<0.01 ASA = Aspirin.

Table 4.7 Effect of the Ethanolic Stem-Bark Extract of *P. erinaceus* on Reaction Time of Rats in the Tail Immersion Test

	Mean reaction time (sec)					
	Time (min).					
Treatment *i.p.*	15	30	45	60	75	90
N/saline	2.33 ± 0.33	2.67 ± 0.33	2.33 ± 0.33	2.43 ± 0.33	2.33 ± 0.33	2.33 ± 0.33
P. e. 50mg/kg	3.33 ± 0.88	4.00 ± 0.00**	5.00 ± 0.57**	5.33 ± 0.66**	5.00 ± 0.58**	3.33 ± 0.88
P.e 100mg/kg	3.67 ± 0.67	2.33 ± 0.33	5.33 ± 0.67**	5.33 ± 0.88**	4.33 ± 0.33**	2.33 ± 0.33
P. e 200mg/kg	2.33 ± 0.33	4.67 ± 0.33**	5.67 ± 1.20*	5.00 ± 1.00*	3.33 ± 0.33	2.66 ± 0.33
Pentazocine 5mg/kg	3.00 ± 0.58	7.33 ± 0.66**	6.67 ± 0.33**	6.33 ± 0.66**	4.00 ± 0.58*	2.67 ± 0.33

n = 5 * P<0.05 ** P<0.001

4.5 **Anti-inflammatory Studies.**

Sub - plantar injection of fresh egg albumin produced a local edema reaching its maximum at about 60min after injection. Ethanolic stem-bark extract of *P. erinaceus* (50,100 and 200mg/kg) and piroxicam (5mg/kg) produced significant ($P<0.01$-0.001) reduction in the egg albumin -induced oedema over a period of 120min time - dependently. Piroxicam (5mg/kg) was however observed to be more effective than the extract in reducing the oedema throughout the duration of the study except at 20min when 200mg/kg extract was more effective then piroxicam.(Table 4.8).

The ethanolic stem bark extract of *P.erinaceus* (50 – 200mg/kg) gave a dose dependent increase in percentage of inhibition (42.23-44.14%) of oedema in rats' hind paw. However the highest percentage of inhibition (58.6%) was observed with piroxicam (Table 4.9).

Table 4.8. Effect of *P. erinaceus* extract and Piroxicam on Egg albumin-induced oedema in Rats.

	Paw volume (ml) at various times (min						
Treatment i.p	0	20	40	60	80	100	120
Normal saline	0.31±.02	0.44 ± 0.03	0.38± .07	0.45±.08	0.39±.06	0.39±0.04	0.36± .03
P.e 50mg/kg	0.18±.02	0.27±.02**	0.28±.03	0.20±.02*	0.19±.01**	0.19±0.04**	0.25± .02
P.e 100mg/kg	0.15±.02	0.33 ± 0.04	0.26±.03	0.22±.03*	0.20±.01*	0.21±0.05*	0.21±.02**
P.e 200mg/kg	0.16±.02	0.21 ± 0.03	0.26±.02	0.27±.05	0.20±.03*	0.22±.03**	0.20±.01***
Piroxicam 5mg/kg	0.15±.03	0.22 ± 0.02	0.20±.02	0.16±.02**	0.13±.02**	0.12±.01***	0.14±.05**

n = 6; P *<0.01 P **<0.001

Table 4.9. Percentage Inhibition of Oedema in Rat Paws by the Ethanolic Stem-Bark Extracts of *P.erinaceus*

	Percentage inhibition of Oedema (%)							
I.P treatment	O	20	40	60	80	100	120	Mean % inhibition
P. e 50m/kg	41.9	38.6	26.3	55.6	51.3	51.3	30.6	42.23 ± 4.21
P.e 100mg/kg	51.6	25.0	81.6	51.1	48.7	46.2	41.7	42.27 ± 3.89
P.e 200mg/kg	48.4	52.3	31.6	40.0	48.7	43.6	44.4	44.14 ± 2.59
Piroxicam 5mg/kg	51.6	50.0	47.4	64.4	66.7	69.2	61.1	58.60 ± 3.33

4.6 **Effect of Extract on Pregnant Rat Uterus**

The extract at 4µg/ml to 3.2mg/ml doses did not produce any effect on the pregnant rat uterus. However, full relaxation was observed at 6.4mg/ml dose. The extract also attenuated contractions produced by 0.008 iu/ml Oxytocin dose - dependently (Fig.4.1 & 4.2).

4.7 **Effect of Extract on Guinea Pig Ileum**.

P. erinaceus extract (8ug/ml-1.6mg/ml) exerted a relaxant effect dose dependently on the guinea pig ileum. Acetylcholine (0.04-0.32µg/ml) caused a concentration dependent contraction of the guinea pig ileum. *P. erinaceus* (1-50mg/ml) reduced the contractile effect of acetylcholine dose – dependently (Fig.4.3 & 4.4).

Histamine (0.8-3.2µg/ml) also caused a concentration dependent contraction of the guinea pig ileum which was slightly reduced by *P. erinaceus* extract (400-800µg/ml) (Fig.4.4).

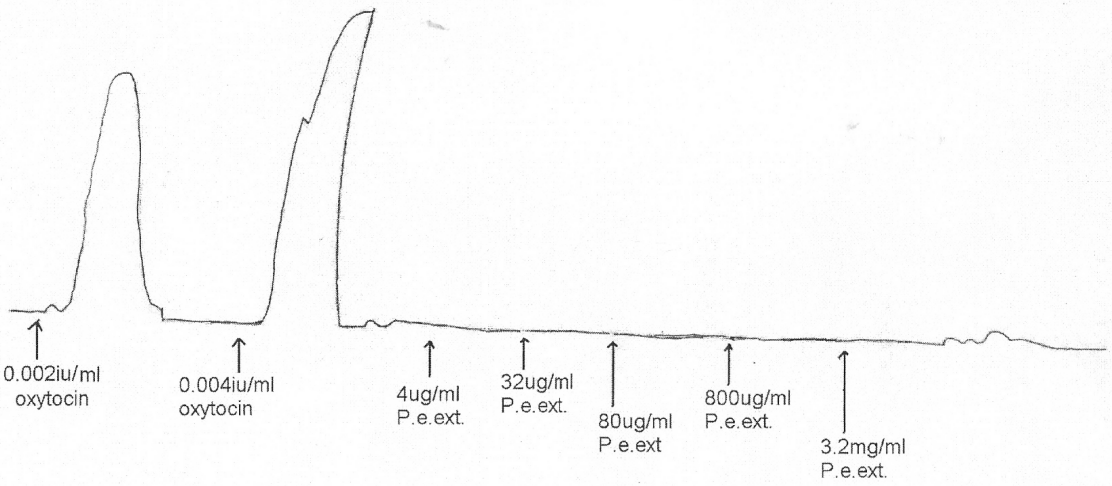

Fig.4.6; Effect of ethanolic stem bark extract of P.erinaceus on pregnant rat uterus.

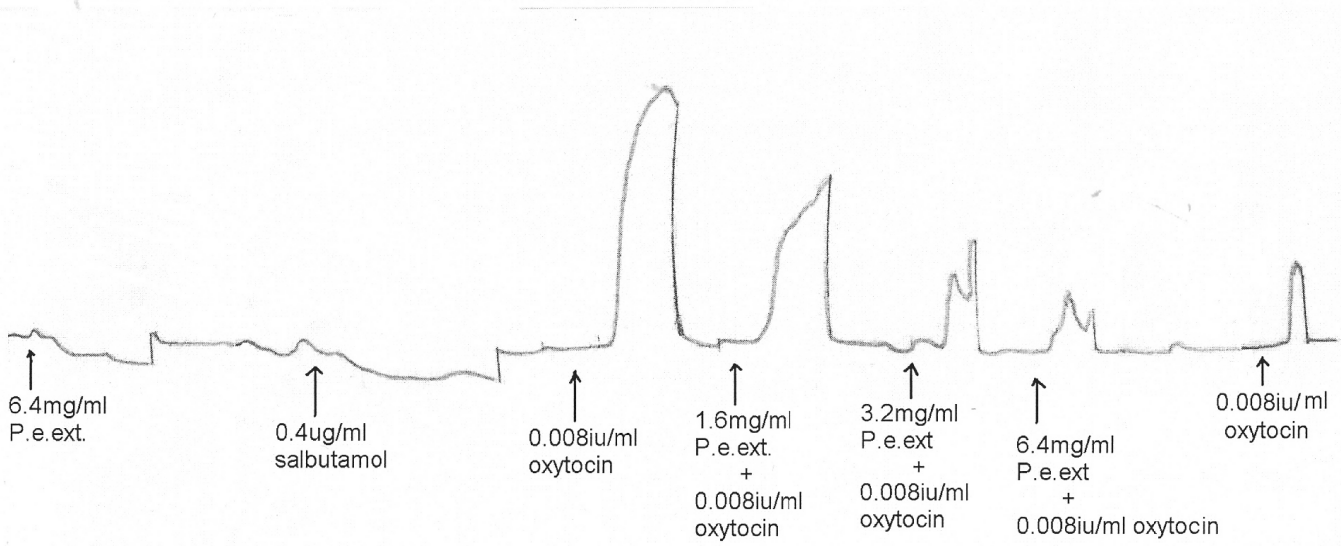

Fig.4.7; Effect of ethanolic stem bark extract of P.erinaceus on pregnant rat uterus.

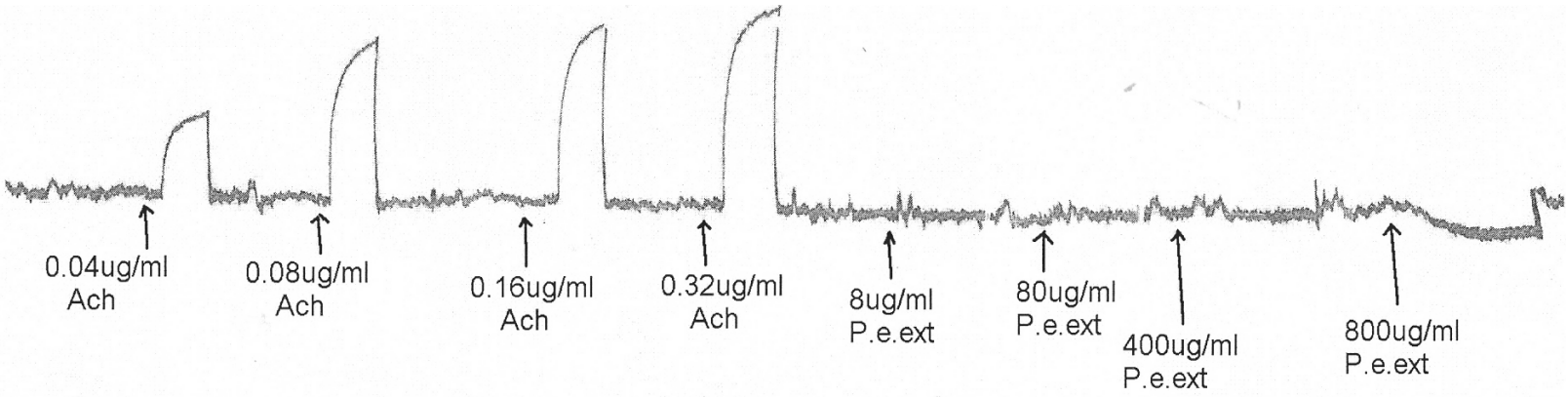

Fig.4.8 Effects of ethanolic stem bark extract of P.e on guinea pig ileum

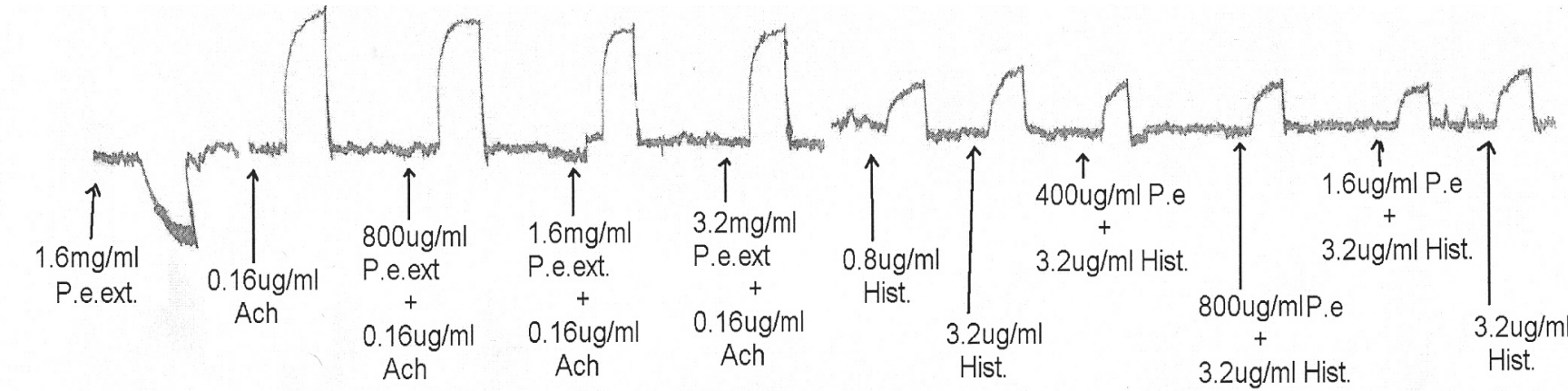

Fig.4.9; Effect of ethanolic stem bark extact of P.erinaceus on Guinea pig ileum.

4.8 **Effect of Extract on isolated Rabbit Jejunum**

Acetylcholine (0.0004-0.0032ug/ml) caused a concentration dependent contraction of the rabbit jejunum, while the extract (0.8-3.2mg/ml) produced a dose dependent inhibition of the spontaneous contraction of the jejunum (Fig.4.5). The extract attenuated acetylcholine induced contraction of the rabbit jejunum concentration dependently (Fig.4.6).

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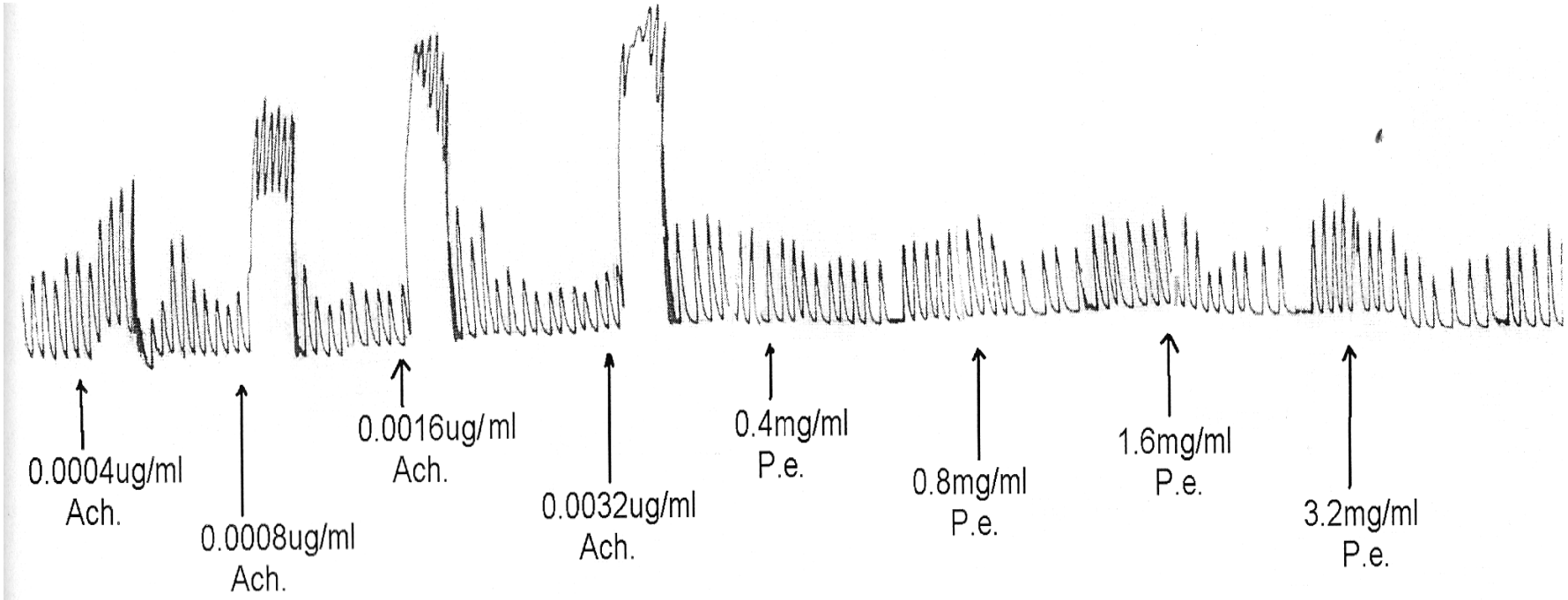


Fig.4.10;Effect of ethanolic stem bark extract of P.erinaceus on rabbit jejunum.

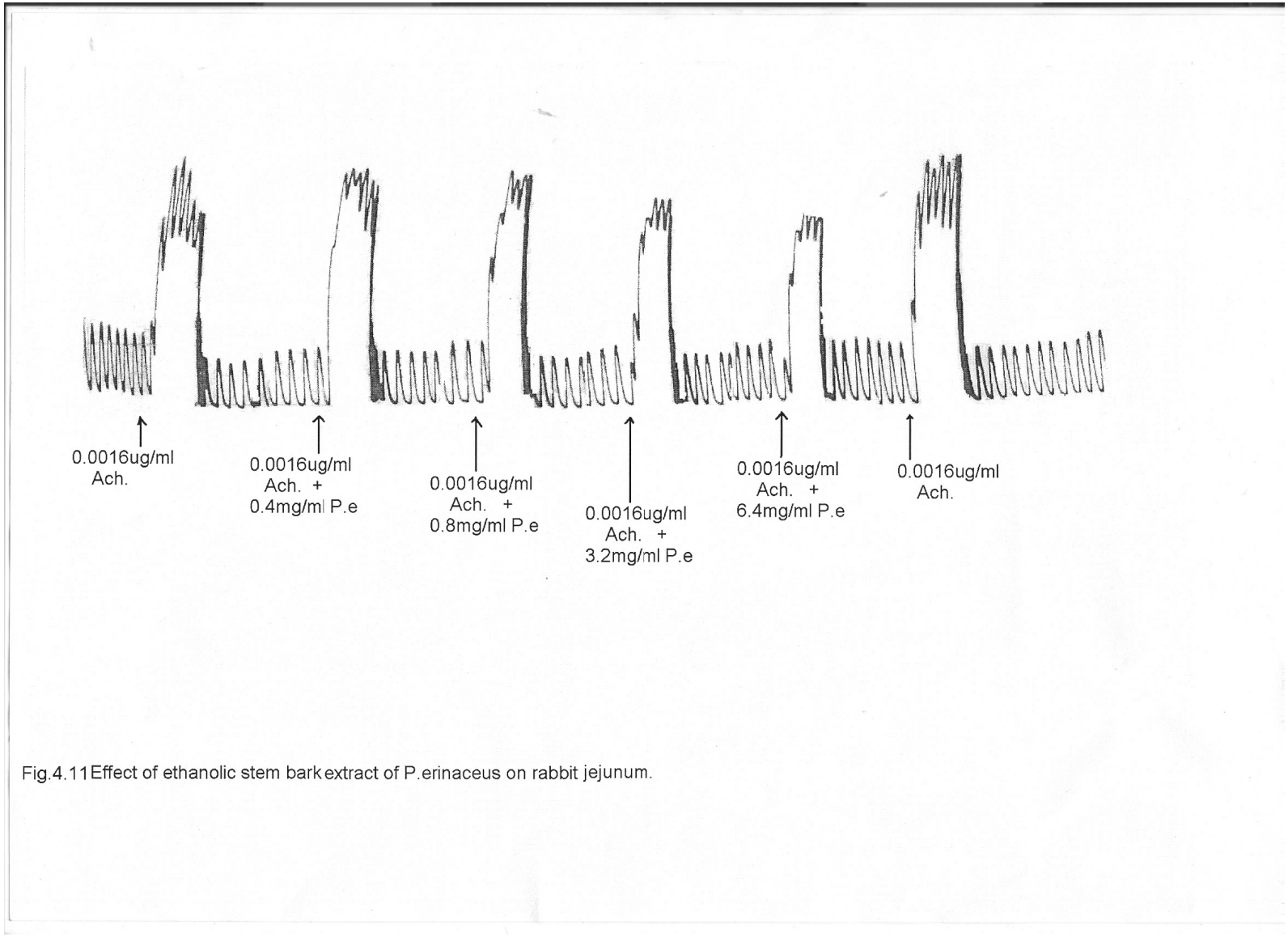


Fig.4.11 Effect of ethanolic stem bark extract of P.erinaceus on rabbit jejunum.

CHAPTER 5
DISCUSSION

Medicinal plants are known to possess various phytochemical principles, which are responsible for their pharmacological activities and some side effects.

Single plant material usually contains diverse chemical constituents that are responsible for their diverse pharmacological activities. Similarly, the presence of the chemical substances may also be responsible for the numerous side effects of these plants.

The result of this study shows the presence of carbohydrates, tannins, flavonoids, steroids, proteins and amino acids in the ethanolic stem bark extract of *P.erinaceus* which are responsible for its pharmacological actions. Alkaloids and saponins were however absent.

Assessment of acute toxicity of an unknown substance is the first step in the toxicological investigation. The most frequently used acute toxicity test involves the determination of median lethal dose (LD_{50}). The LD_{50} was defined by Gehring et al (1973) as "a statistically derived expression of a single dose of a material that can kill 50 percent of the animals". The value represents the amount of toxicant per unit weight of the animal, which will kill 50 percent of the particular population of the animal species employed for the tests within a specific period of time, usually 24-72 hours. The routes of administration and animal species used affect the mean lethal dose. A scale proposed by

Lorke, (1983) roughly classifies substances according to their LD_{50} as follows: very toxic (LD_{50} < 1.0mg/kg), toxic (LD_{50} up to 10mg/kg), less toxic (LD_{50} up to 100mg/kg), only slightly toxic (up to 1000mg/kg) and substances with LD_{50} values greater than 5,000mg/kg are practically non toxic. This justifies the traditional use of this plant orally for the treatment of various diseases like fever (Irvine, 1961), diarrhea and dysentery (Dalziel, 1948) without harmful effects.

Matsumura (1975) and Corbett et al. (1984) have also classified chemicals according to their oral LD_{50} values as follows: Extremely toxic $LD_{50} \leq$ 1mg/kg, highly toxic; 1-50mg/kg, moderately toxic; 50-500mg/kg, slightly toxic; 0.5-5g/kg, practically non toxic; 5-15g/kg, relatively harmless; > 15g/kg. This categorization though convenient is only a rough guideline.

The intraperitoneal LD_{50} of 447.21 mg/kg of the ethanolic stem bark extract of *Pterocarpus erinaceus* shows that it is slightly toxic, while the oral LD_{50} of › 5000mg/kg revealed that the extract is practically non-toxic by this route.

Coagulation studies are of great importance considering the role of blood in life (Aballi and de-lameran's, 1962). Platelets are the blood cells involved in coagulation (Williams and Levine, 1982). Blood coagulation requires that platelet be in sufficient size, number and function. In experimental animals, it has been reported that the bone marrow has a narrow reserve of platelets (Burstein et al., 1981). Prolonged bleeding time and clotting

time in the presence of a reduced platelet count is indicative of a general platelet insufficiency to form haemostatic plugs.

Vascular resistance to injury are measured by capillary fragility and bleeding time. Factors that affect capillary fragility include hereditary haemorrhagic telangiectasia (where vessel walls consist of only one endothelial layer) and scurvy (vitamin-C) deficiency. Vitamin C is necessary for the synthesis of cement substances that link the epithelial cells of the capillary walls.

Pterocarpus erinaceus ethanolic extract did not affect the Haemoglobin concenration, packed cell volume, white blood cell count, clotting time and platelet count in the treated animals, suggesting that it does not exert its haemostatic effect through the blood cells. The bleeding time was however significantly reduced ($P < 0.05$) at the dose of 200mg/kg, which might suggest its possible activity on the integrity of blood vessels to reduce bleeding.

Since none of the rats used in this study died, this could suggest minimal cumulative adverse effects after chronic administration of the extract.

Flavonoids like quercetin and rutin have been used as effective constituents of several pharmaceuticals used for treatment of capillary fragility and phlebosclerosis.(Catherine *et al.*1996). It has been suggested that flavonoids, which contain free hydroxyl groups at 3, 3’ and 4’ positions exert beneficial physiological effects on capillaries. Orally

administered flavonoids have been observed to inhibit vascular permeability and prevent pulmonary haemorrhage. Acacatin at 25-100mg/kg administered orally to mice reduced capillary fragility and at 50-100mg/kg doses, reduced vascular permeability (Wenner *et al*.1980). Flavonoids play this important role of maintaining capillary integrity by functioning as antioxidants (Frei *et.al.,* 1989).

Plants with high tannin concentration like *Rhus semialata, Punica granatum* and *Cedrela sinensis* have also been used to arrest functional bleeding by the Chinese (Subhuti, 2003).

The haemostatic property of the ethanolic extract of *P.erinaceus* is likely to be due to the flavonoids and tannins identified during phytochemical screening.

Pain is a signal indicating tissue damage or physiological malfunction. Stimulation of peripheral pain receptors transmits impulses through pain pathways to the brain. Pain is a pathological condition resulting from the release of a variety of chemical substances that activate directly or enhance the sensitivity to other forms of stimulation.

Bradykinin belongs to the group of autacoids called polypeptide kinins. Other polypeptides in this group are kallidin, angiotensin and substance P. When exposed to sensory nerve endings in human skin, bradykinin causes pain.

The inhibitory effect of aspirin and other NSAIDS on prostaglandin synthetase implies that prostaglandins are mediators of pain inducing stimuli. However prostaglandins do

not act directly to stimulate sensory receptors subserving pain, but they lower the threshold to stimulatory substances like bradykinin thereby producing hyperalgesia.

Platelet-activating factor (PAF) has been implicated as a potent lipid mediator of inflammation and allergy. In inflammatory cells, PAF is synthesized via a remodeling pathway in which arachidonic acid is released and metabolized by cyclooxygenase and lipooxygenase to prostaglandins and leukotrienses respectively.

Pain can also be induced by injection of irritants into the peritoneal cavity of mice. The animal reacts with a characteristic stretching behaviour, which is called writhing.

The acetic acid-induced abdominal writhing method is used to elucidate peripheral analgesic effects. It is a very sensitive method that is able to detect antinociceptive effects at dose levels that are inactive in other methods like tail-flick test (Bentley et al, 1981).

Acetic acid causes release of endogenous substances, which then excite the pain nerve endings producing the characteristic writhing abdominal response.

The abdominal constriction response is postulated to partly involve local peritoneal receptors (Bently et al., 1983) and lipoxygenase products (Levini et al, 1984; Ohara et al, 2000).

The results of acetic acid-induced abdominal constriction test suggest that the analgesic action produced by the extract may be linked to inhibition of both lipooxygenases and cyclooxygenases. Both lipooxygenases and cyclooxygenases are important enzymes in the biosynthetic pathway of prostaglandins which are mediators of inflammation and pain. This suggests that the effect of the extract on peripherally induced pain is linked to inhibition of prostaglandin synthesis.

Results of the tail immersion method showed that the extract raised the pain threshold by increasing reaction time to thermal nociceptive stimulation. Chou (1989) proposed that thermal painful stimuli are selectively alleviated by centrally acting analgesic drugs. Pentazocine is a partial opioid agonist used therapeutically in the treatment of moderate pain. The extract is however less potent than pentazocine in this model.
The antinociceptive activity of the extract may therefore be both peripherally- and centrally- mediated.

Anti inflammatory drugs are mainly screened based on their ability to inhibit the oedema produced in the hind paw of the rat after injection of phlogistic (oedema-inducing) agent (Vogel and Vogel, 1997). The oedema caused by different irritants lasts for different times ranging from a few hours with serotonin and egg albumin, to up to 2 days with aerosil or kaolin. The paw oedema method has proven to be suitable for detecting activity in acute inflammation as well as for more in-depth evaluation (Vogel and Vogel, 1997). The extract caused a marked reduction in albumin-induced hind paw oedema in the rats.

Aspirin and other NSAIDs have analgesic and antipyretic activity in addition to their anti-inflammatory activity. They are effective in preventing the oedema, cellular exudates and pain of experimental inflammation. They act by inhibiting the biosynthesis of prostaglandins, which are important mediators of the inflammatory reaction.

Flavonoids are known to target prostaglandins in the late phase of acute inflammation and pain perception (Rajnarayana et al., 2001). Several flavonoids isolated from medicinal plants have been shown to posses significant anti-nociceptive and/or anti-inflammatory effects (Duke, 1992). It is therefore; possible that both the anti-nociceptive and anti-inflammatory effects of this extract may be attributable to its flavonoid component as shown in the phytochemical analysis.

Acetylcholine binds to muscarinic receptors on ileal smooth muscles causing the receptor-operated channel to open thus allowing sodium influx, which causes depolarization of the cell membrane. This depolarization opens voltage- dependent calcium channels and calcium ions enter the cell to induce the release of calcium from sarcoplasmic reticulum. The cytosolic calcium then binds to calmodulin and contraction is produced (Bolton, 1979).

Histamine binds to H_1 receptor on gastrointestinal smooth muscle to initiate the same sequence of events (Bohr, 1973).

An elevation of intracellular Ca^{2+} level by influx from extracellular compartment or release from intracellular store also results in contraction. (Ebeigbe, 1982).

Slight inhibition of acetylcholine and histamine-induced contraction of the rabbit jejunum and guinea pig ileum shows that the extract interacts with both muscarinic and histaminic receptors.

The extract could possibly prevent calcium influx through the voltage operated channels by inhibiting the calcium induced calcium release mechanism preventing the release of calcium from the sarcoplasmic reticulum or preventing binding of calcium to calmodulin. This activity could be linked to the presence of tannins and flavonoids that have been used in the treatment of diarrhea and dysentery.

The uterine smooth muscle is a mass of structures such as nerves and connective tissues. Its responsiveness to drugs is markedly influenced by hormonal and physical factors such as pregnancy and oestrous cycle. It also varies from species to species. The uterus has parasympathetic and sympathetic innervations.

Many drugs have the capacity to stimulate the uterine smooth muscles. However, only a few have effects that are sufficiently selective and predictable to justify their use as uterine stimulating agents in obstetric practice. These are oxytocin, certain prostaglandins and ergot alkaloids (ergometrine and methylergometrine). Each in appropriate doses during pregnancy is capable of eliciting gradual increase in uterine motility from

moderate increase in the rate and force of spontaneous motor activity to sustained titanic contractions, while causing minimal side effects in healthy subjects.

Oxytocin, a neurohypophyseal polypeptide released by posterior pitutuary stimulates both frequency and force of contraction of uterine smooth muscles.

The action of oxytocin on myometrium is independent of innervation. There are specific oxytocin receptors, which mediate the response mainly by depolarization of muscle fibres, influx of calcium ions and intracellular release of calcium ions. The number of oxytocin receptors increase markedly in late pregnancy.

The ethanolic bark extract of *Pterocarpus erinaceus* produced dose dependent relaxation of the smooth muscle of the isolated pregnant uterus of rat. Oxytocin induced contraction was also blocked by the ethanolic extract. Indomethacin, which inhibits prostaglandin synthesis, has been observed to antagonize oxytocin-induced contractions (Kitchen, 1984). This suggests that endogenous prostaglandin may contribute to the stimulant activity of oxytocin. The ethanolic bark extract of *Pterocarpus erinaceus* may thus be producing inhibition of uterotonic effect by inhibiting prostaglandin synthesis thus reducing the concentration of endogenous prostaglandins (E_2 & $F_2\alpha$) necessary to produce uterotonic action.

Prostaglandins have complex effects in relation to adenyl cyclase and cyclic AMP. They stimulate adenyl cyclase resulting in increase in cAMP.

(Revuelta *et al.,* 1999) reported that flavonoids such as genistein, quicetin produced relaxation of the uterine smooth muscle by increasing cAMP.

Since the *in- vitro* studies conducted during this work did not support the ethno-medical use of *P.erinaceus* as part of abortifacient prescription, this may suggest that the plant may not be the active abortifacient constituent of the prescription.

Chapter 6

CONCLUSIONS & RECOMMENDATION

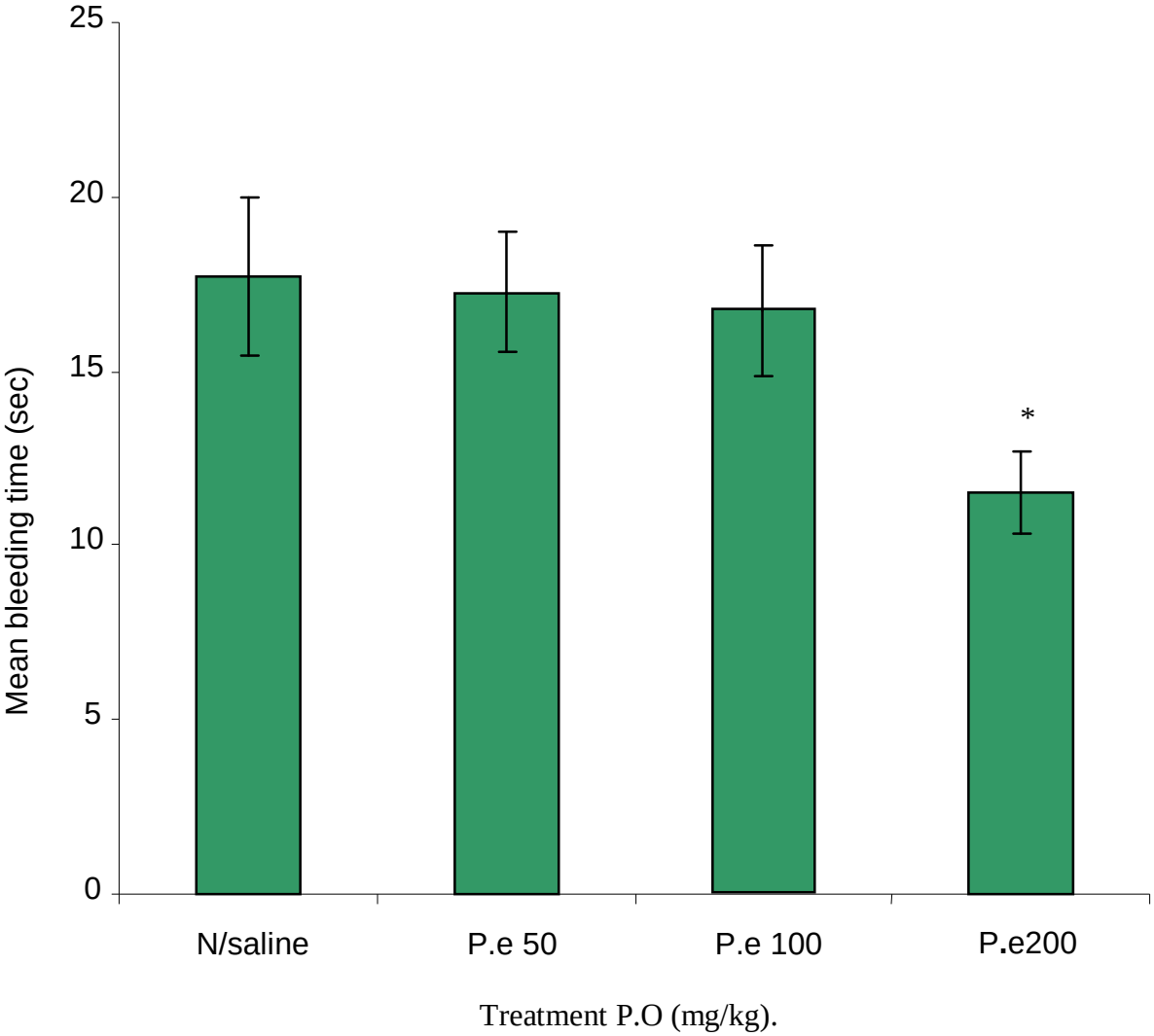
The ethanolic stem bark extract of *Pterocarpus erinaceus* possessed significant analgesic and anti-inflammatory activities. This may partly justify the traditional use of the plant in the treatment of fever and headache.

The extract relaxed smooth muscles of guinea pig ileum and rabbit jejunum *in –vitro*. This probably confirms the effectiveness of the extract in the treatment of diarrhea and dysentery by traditional healers.

The extract relaxed the pregnant rat uterus, which is not in conformity with its claimed use as an ingredient in abortifacient preparations traditionally.

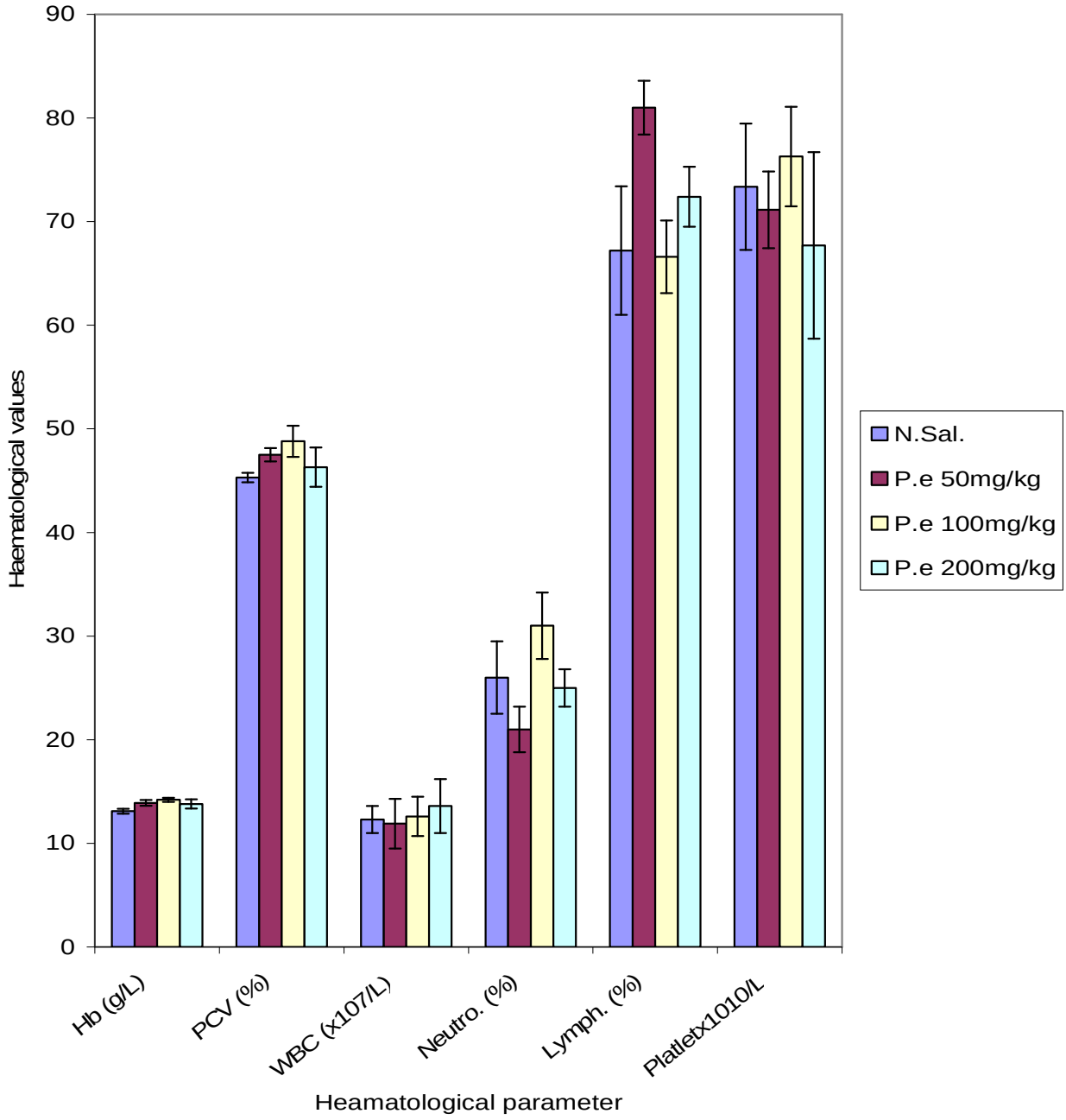
The extract decreased bleeding time in the treated rats; this property lends pharmacological credence to the suggested folkloric use of *Pterocarpus erinaceus* stem bark in the control of nose bleeding and menorrhagia in some communities in Kano, Nigeria. The plant could be of potential benefit as a natural haemostatic agent.

It is recommended that further work be conducted on this plant to reveal other potential benefits that could be tapped as well as isolating the component that may be responsible for pharmacological activity. Investigations on the possible mechanisms of action of the plant could also be conducted.

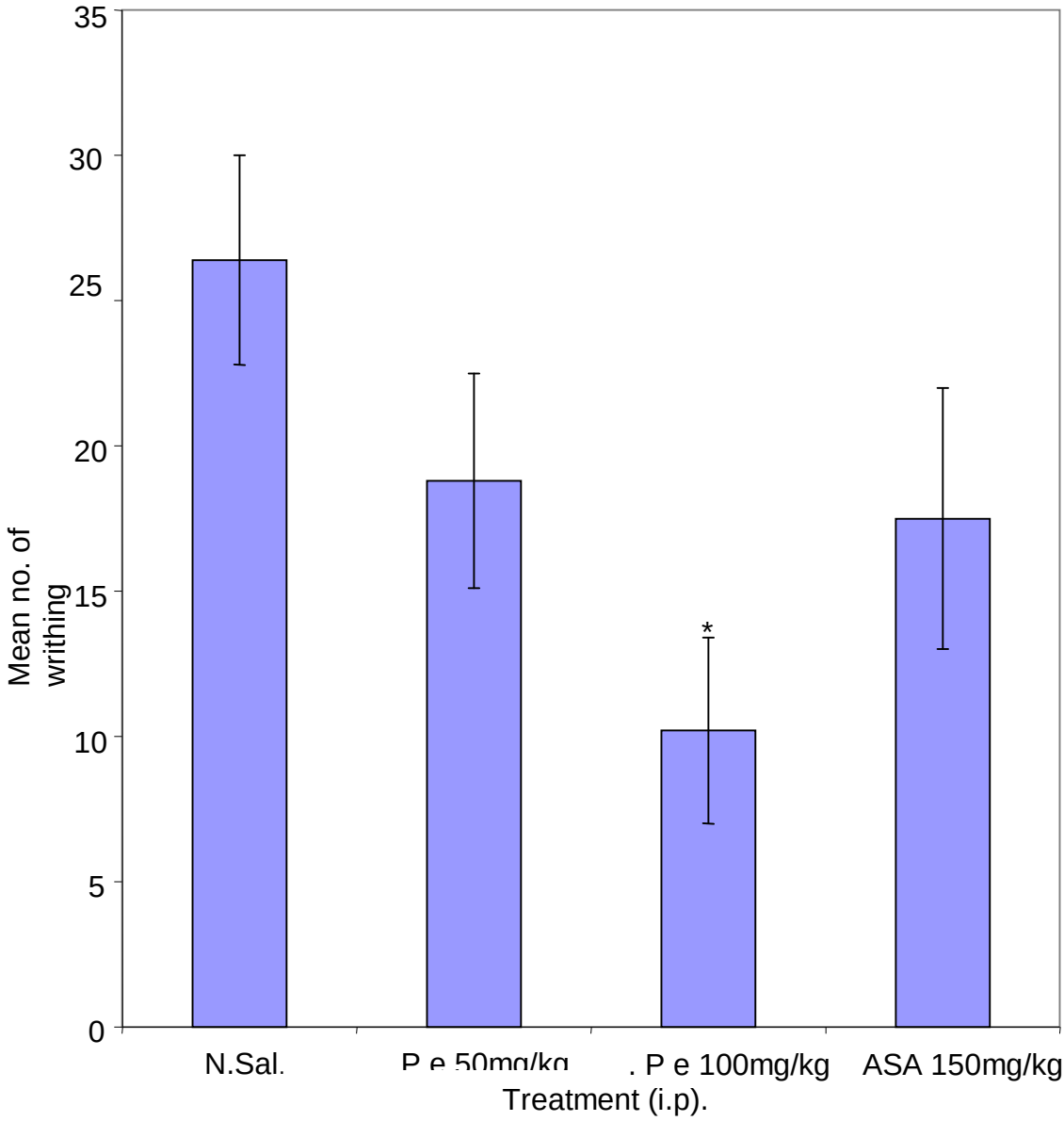


n =5 * significantly different from control at P<0.01

Appendix I. Effect of *P.erinaceus* extract on Bleeding Time of Rats.

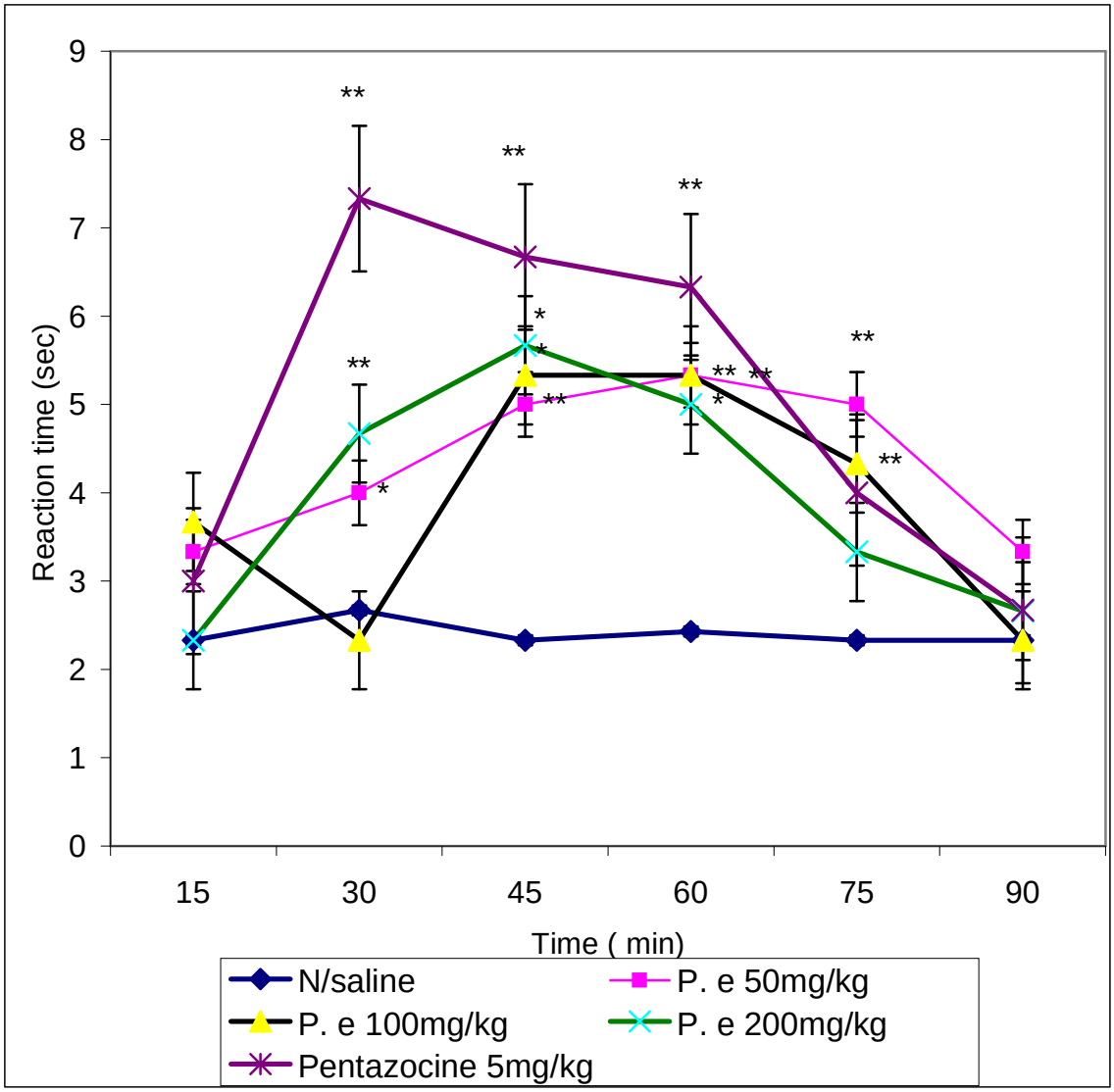


Appendix II. Effect of 30days Administration of *P.e* extract on Hematological Parameters



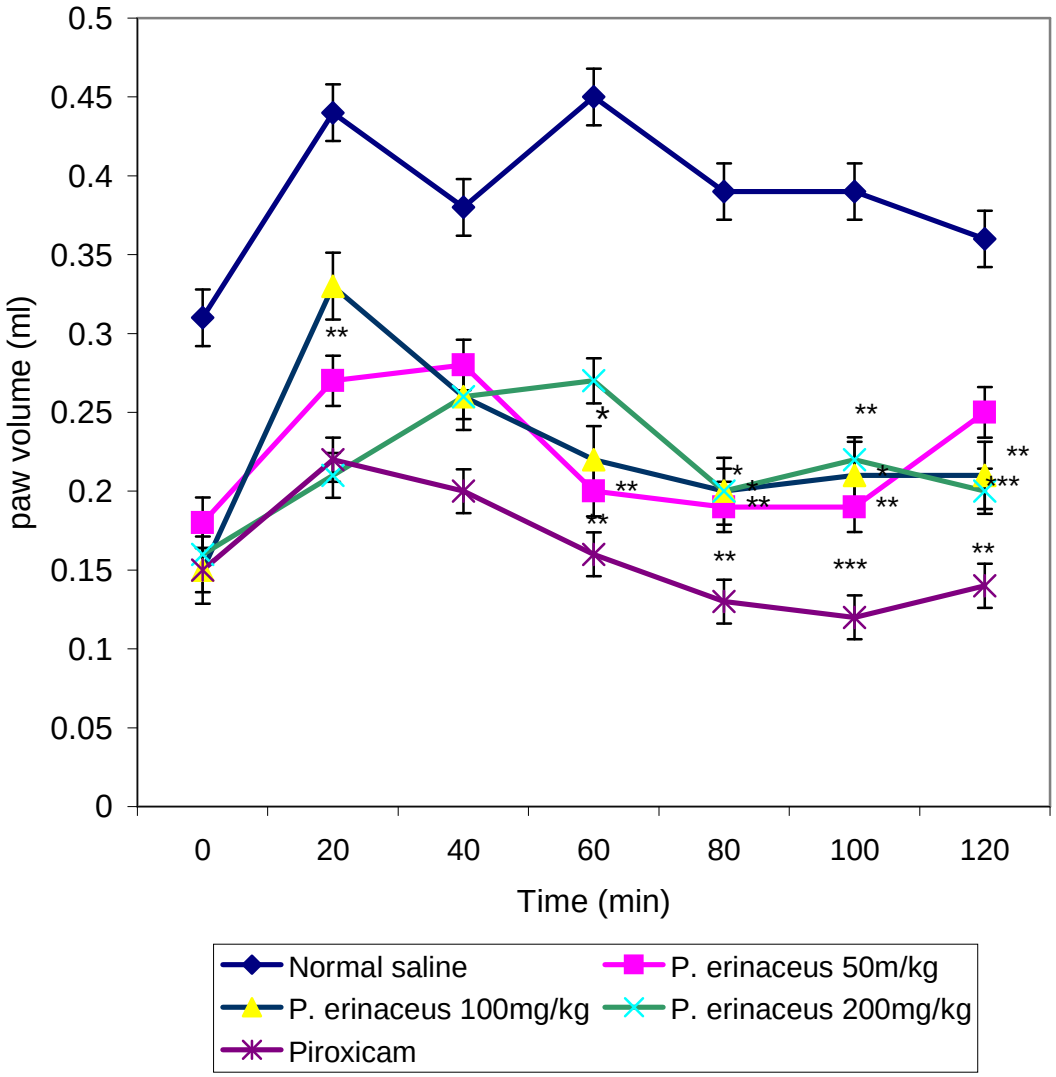
n=5 * P<0.01, compared to control for all values; student's t test;

Appendix III. Effects of Ethanolic stem bark extract of *P.erinaceus* on acetic acid-induced writhing in mice.



n = 6; P < 0.05, compared to control for all values; student's t test;

Appendix IV Effects of Ethanolic Stem-bark Extract of *P.erinaceus* and Pentazocine on Reaction Time (tail removal) of Rats to Heat Stimulus in the Tail Immersion Test.



n=6; P *<0.01, P **<0.001,

Compared to control for all values; student's t test;

Appendix V Effect of Stem Bark Extract of *P.erinaceus* on Egg Albumin Induced Paw Oedema in Rats.

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