1. General Introduction

1.1 Platelets:

1.1.1History

Platelets, clinically known as Thrombocytes, are small components of blood produced in the bone marrow with a key role in blood clotting. The major function of thrombocytes is to stop bleeding from injured blood vessels. These anucleated cell fragments in mammals, are called as platelets (Hawkey, 2013). The non-mammalian vertebrate thrombocytes differ from the mammalian platelets in the presence of nucleus and they do not aggregate in response to ADP, serotonin and adrenaline. Platelets were first observed by Leewenhoeck at the Royal Society of London (Van Leeuwenhoek, 1674). Though Hewson (1771) had fully described platelets them for the first time in 1780. He had described them as minute, undefined particles in blood. Alfred Donne, a histologist from France, later named them in 1842 as "globulin du chyle" (that is small globules derived from plasma) a sort of small globular, pale, opaline corpuscles visible in blood (Donne, 1842). The same corpuscles were later described by Beale in 1850 as particles of "germinal matter" (Bioplasma kornchen) and by Zimmermann in 1860 as "small corpuscles" (Bizzozero, 1881, 1883). Bizzozero (1881), who named it "piasthne", was the first one to clearly establish the significance of these particles that were visible not only in blood extracted from veins, but also in circulating blood. Finally, the first careful description of these blood components was made by Schultze (1865). He described them as clumps of irregular shape structure with different sizes ranging up to 80 µm and filled with small 1-2 µm globules or colorless granules.

1.2 Biogenesis of Platelets:

Platelets, the coagulating corpuscles of the blood, are formed and released into the bloodstream by precursor cells called megakaryocytes that reside within the bone marrow. Production involves a complex series of remodeling events which results in the release of thousands of platelets from a single megakaryocyte. Thereby, defect in this process can result in clinically significant disorders or conditions known as Thrombocytopenia (platelet counts less than 150,000/µl) or Thrombocythemia (platelet counts greater than 600,000/µl). Thrombocytopenia can lead to inadequate clot formation and increased risk of bleeding while Thrombocythemia can heighten the risk for thrombotic events, including stroke, peripheral ischemia, and myocardial infarction. The formation of platelets occurs via essential intermediate pseudopodial extensions called proplatelets. Proplatelets are generated by the outflow and evagination of the extensive internal membrane system of the mature megakaryocytes. Consequently platelet fragments from the megakaryocyte extensions are release into the blood vessels supplying bone marrow (Radley & Haller, 1982).

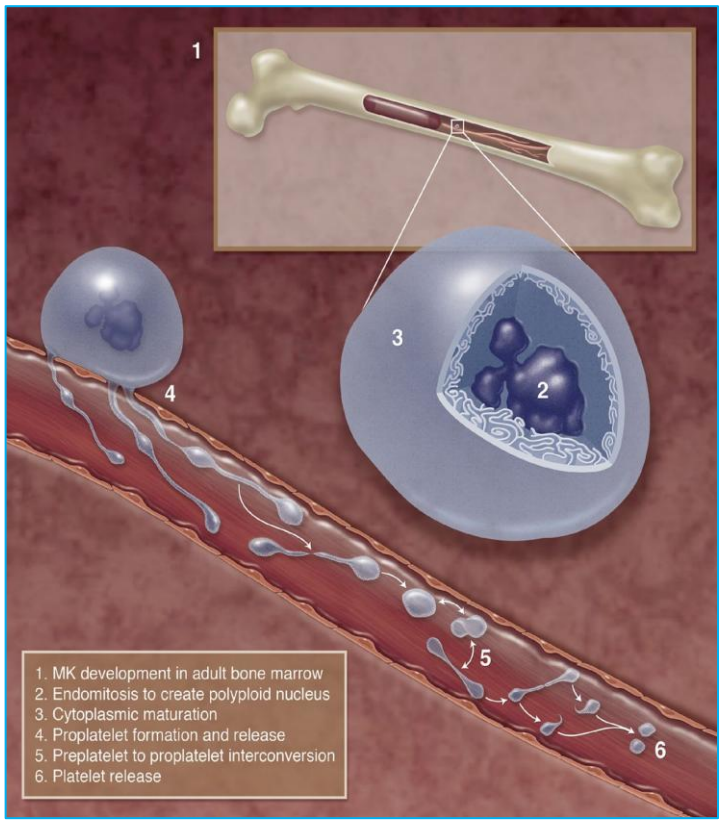

Fig 1.1: Platelet formation release in the blood

JCB Home » 2013 Archive » 10 June » 201 (6): 785, Review. The incredible journey: From megakaryocyte development to platelet formation. Kellie R. Machlus, Joseph E. Italiano. DOI: 10.1083/jcb.201304054 | Published June 10, 2013.

The major events taking place in formation of Megakaryocytes:

- The Megakaryocyte enlarges considerably in size to approx 100 µm in diameter.
- The enlargement of the cell is mediated by numerous rounds of endomitosis directed by thrombopoetin (Ebbe, 1976).
- Endomitosis causes amplification of DNA as much as 64 fold (sometimes even 128 N).
- Nuclear envelop vanishes, interconnected mitotic spindles assemble and the mitotic cycle arrests at anaphase.
- The spindles fail to separate and telophase and cytokinesis are bypased.

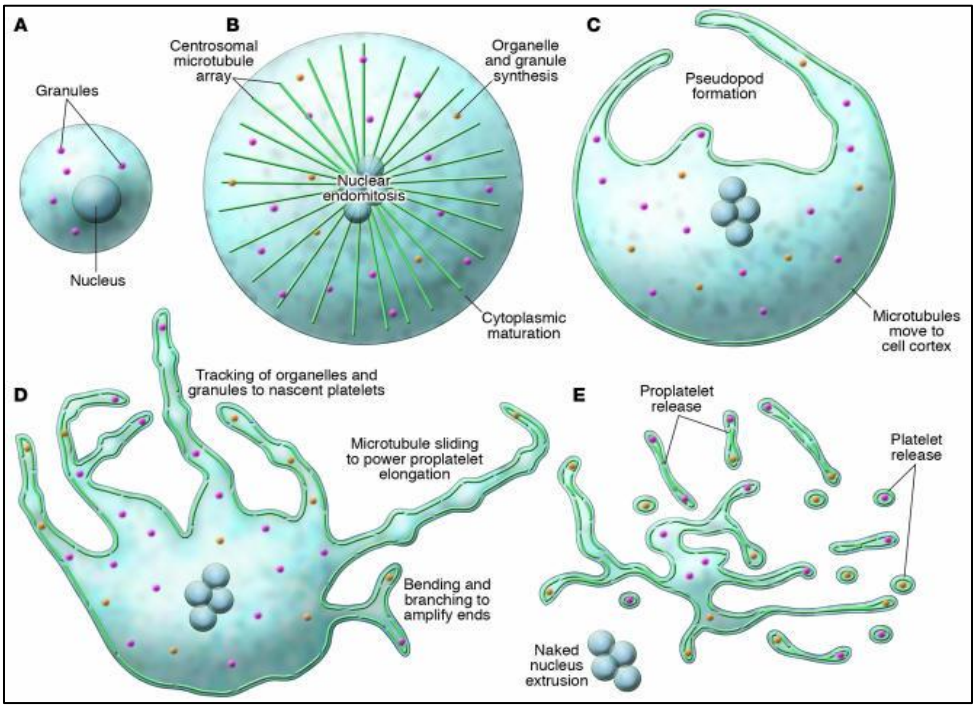

Fig 1.2: Overview of megakaryocyte production of platelets
Citation Information: J Clin Invest. 2005;115(12):3348-3354. doi:10.1172/JCI26891. https://www.jci.org/articles/view/26891

- Nuclear envelope is reformed and the cell is now polyploid (4N to 128N) (Odell *et al.*, 1970).
- Assembly of large number of ribosomes to facilitate the production of platelet specific proteins occurs (Long *et al.*, 1982).
- In addition to expansion of DNA, significant maturation occurs as the internal membrane systems, granules and organelles are assembled in bulk.
- In particular, there is formation of an expansive and interconnected membranous network of cisternae and tubules called the demarcation membrane system (DMS).
- Finally, the membrane of mature megakaryocyte evaginates and platelets fragments from the end of megakaryocyte extensions are released into the blood vessels supplying bone marrow.

1.3 Stucture of platelets:

Blood is a complex liquid tissue containing broadly three different types of corpuscles viz. Erythrocytes, Leucocytes and Platelets. Unlike red and white blood cells, platelets are not actually cells but rather small fragments of cells nearly ¼ size of RBC.

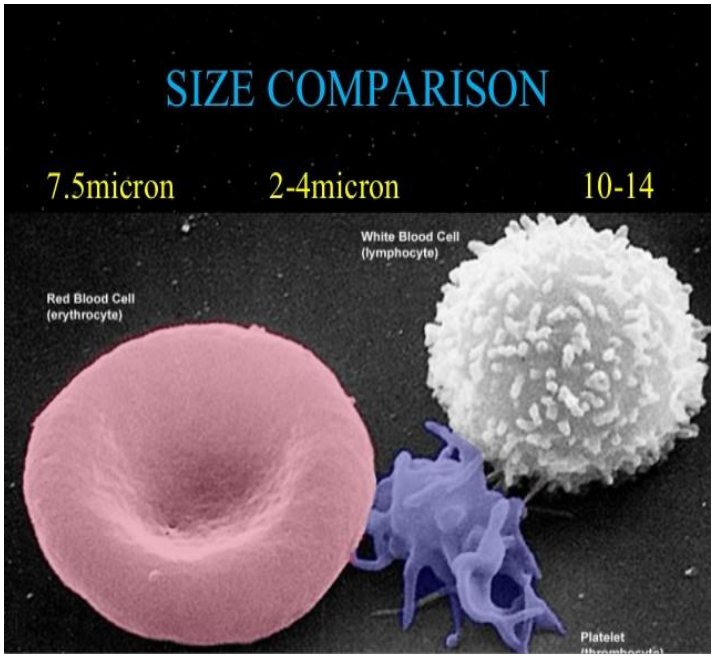

Fig 1.3: Size Comparison of Blood Corpuscles

https://embryology.med.unsw.edu.au/embryology/images/6/66/Erythrocyte_and_lymphocyte_SEM02.jpg

Ultrastructurally, the platelet can be divided into four zones, from peripheral to innermost:

- Peripheral zone - is rich in glycoproteins required for platelet adhesion, activation, and aggregation. For example, GPIb/IX/X; GPVI; GPIIb/IIIa.
- Structural (Sol-gel) zone - is rich in microtubules and microfilaments, allowing the platelets to maintain their discoid shape.

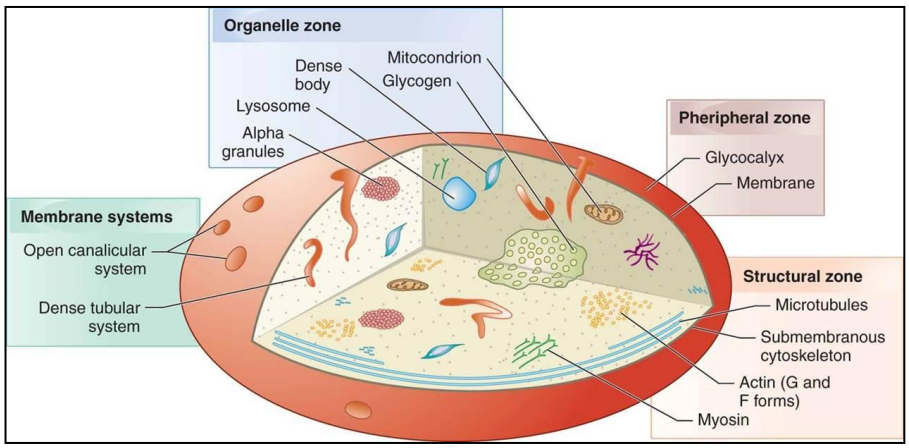

Fig 1.4 : Diagram representing structure of Platelets

Citation information: http://passtheclassandnotfail.blogspot.in/2013/03/primary-hemostasis.html

- Organelle zone - is rich in two types of granules. 1) Alpha granules that contain clotting mediators such as factor V, factor VIII, fibrinogen, fibronectin, platelet-derived growth factor, and chemotactic agents. 2) Delta granules, or dense bodies, containing ADP, calcium and serotonin, the platelet-activating mediators.
- Membranous zone - contains membranes derived from megakaryocytic smooth endoplasmic reticulum organized into a dense tubular system which are responsible for thromboxane A2 synthesis. This dense tubular system is connected to the surface platelet membrane to aid thromboxane A2 release.

1.4 Platelet Disorders:

Platelet disorders can be divided into Qualitative and Quantitative disorders.

1.4.1 Qualitative Platelet Disorders:

Qualitative platelet disorders are suggested by a prolonged bleeding time (abnormal platelet function screen) or clinical evidence of bleeding in the setting of a normal platelet count and coagulation studies. They are usually acquired, but can be inherited. A new platelet function test, PFA-100 (Dade-Behring, Deerfield, Ill), has a 96% sensitivity for detecting platelet disorder like von Willebrand disease and aspirin-induced platelet defects (Mammen *et al.*, 1998). It has yet to find a place among routine coagulation laboratory tests.

i. Drug-Induced Platelet Dysfunction

The most common drug responsible for this dysfunction is aspirin, which irreversibly inhibits cyclooxygenase and blocks the formation of Thromboxane A2 (Loll *et al.*, 1995). Other common drugs include clopidogrel, ticlopidine, and glycoprotein IIb/IIIa inhibitors. Nonsteroidal anti-inflammatory drugs (NSAIDs), unlike aspirin, bind reversibly at the active site of the enzyme. This binding usually depresses platelet thromboxane formation to the degree that platelet function is impaired for only a portion of the dosing interval (Pedersen & FitzGerald, 1985).

ii. Uremia

Platelet dysfunction, “uremic thrombocytopathy” in renal failure, is attributable to high levels of small, partly dialyzable molecules known as uremic toxins (Boccardo *et al.*,

2004). This imparts a predisposition to bleeding that is incompletely understood. Treatment involves correction of anaemia, hemodialysis, and the use of desmopressin. Platelet transfusions do not correct the coagulopathy because the transfused platelets will assume the dysfunction of the uremic platelets (Weigert & Schafer, 1998).

iii. Liver Disease

Whether acute or chronic, hepatic disease is associated with platelet dysfunction that is multifactorial in origin as liver synthesizes Thrombopoetin and various clotting factors. Increased Fibrin Degradation Product (FDP) levels from activation of the fibrinolytic pathway compromise platelet function and impair release of platelet factor III from platelets because of cirrhosis or manifestations of hepatic dysfunction (Al Ghumlas & AbdelGader, 2003; Wilson *et al.*, 1968).

iv. Acquired von Willebrand Disease

Acquired von Willebrand disease (vWD) is often described in patients with autoimmune disorders, lymphoproliferative disorders, or monoclonal gammopathies. It may also be drug induced (e.g., by dextran or valproic acid). The pathophysiology varies from adherence of vWF to tumor cells to vWF degradation by proteolytic enzymes.

1.4.2. Quatitative Platelet Disorders:

i. Thrombocytosis

Thrombocytosis is defined as a condition where the platelet count goes above the upper limit of the normal range (450 x 10^9/L in adults) (Skoda, 2009). There are mainly two types of thrombocytosis:

- ***Haematological disease including primary thrombocytosis***

Primary thrombocytosis is also referred to as essential thrombocytosis, essential thrombocythaemia and primary thrombocythaemia. It is caused due to myeloproliferative disorder in which there is failure to regulate the production of platelets (autonomous production). The clinical features include a platelet count greater than 600 x 10^9/L, megakaryocyte hyperplasia, splenomegaly and a tendency to both thrombosis and haemorrhage. In these, platelet survival is normal but function is not. Other haematological diseases which cause thrombocytosis are myeloproliferative, myelodysplastic or a combination of both. It includes some leukaemias too.

- ***Secondary or reactive thrombocytosis***

Platelet count is found to increase in response to various stimuli, including systemic infections, inflammatory conditions, bleeding, and tumors, as they are acute-phase reactants (Mantadakis *et al.*, 2008; Vora & Lilleyman, 1993). This exaggerated physiological response to a primary problem such as infection, is called reactive or secondary thrombocytosis, which is a benign form of thrombocytosis. In this case, the increase in platelet production is caused by the trigger factor (eg., infection) induced

release of cytokines (Araneda *et al.*, 2001; Tefferi *et al.*, 1994). It is often found to be a transient reaction which is subsided when the underlying cause is resolved.

ii. Thrombocytopenia.

Thrombocytopenia is defined as a platelet count of less than 150,000/µl. However thrombocytopenia is rarely the cause of bleeding provided the platelet function is normal. However, medical intervention becomes necessary when the count falls below 50,000/µl. The condition is confirmed by examination of a peripheral blood smear. The etiology of Thrombocytopenia can be decreased platelet production (bone marrow depression/precursor depression), increased destruction, sequestration in spleen, or a combination of these causes.

- ***Platelet Underproduction:***

The underproduction of platelet could be due to decreased marrow precursor i.e. megakaryocytes or faulty biogenesis, resulting into lower peripheral blood reticulated platelet count (Kurata *et al.*, 2001). Causes include infections (including HIV), drugs (frequently chemotherapeutic agents or alcohol, other medications like Vancomycin), radiotherapy, vitamin deficiency (e.g., folate, vitamin B_{12}), or marrow infiltration by tumor, storage diseases, or marrow failure syndromes (e.g., aplastic anemia). In addition, the myelodysplastic syndromes are a frequently overlooked group of disorders associated with thrombocytopenia in older adults.

Management of platelet underproduction involves treatment of the underlying condition, use of corticosteroids and supportive platelet transfusions if needed. Recently two types of recombinant thrombopoietin (TPO) have been used in the clinical arena for the

treatment of chemotherapy-induced thrombocytopenia. These include 1) recombinant human thrombopoietin, rhuTPO, and 2) pegylated recombinant human megakaryocyte growth and development factor, PEG-rhuMGDF (Kuter & Begley, 2002). rhuTPO increases the nadir platelet count and reduces the duration of thrombocytopenia. This results in decrease in platelet transfusion for patients receiving dose-intense chemotherapy for cancer (Kuter & Begley, 2002). Nevertheless, these agents remain the subject of investigation and recommendations as their use have not been defined.

- ***Platelet Sequestration***

About 1/3rd of Platelet population is sequestered in spleen. Hypersplenism, from a variety of causes including liver disease or malignancy, are likely to result in increased platelet sequestration, thereby leading to mild to moderate thrombocytopenia and splenomegaly. Normally Splenomegaly is detected by physical examination. However, when detection is inadequate, evaluation with ultrasonography or radionuclide imaging is recommended to document splenomegaly. The main causes for spleenomegaly is believed to be Cirrhosis of liver, Heart failure, Portal or hepatic venous thrombosis, Malignancies and hematologic disorders, including lymphoma, acute and chronic leukemias, myeloproliferative disorders, metastatic solid tumors and hemolytic anemias. Infection with Epstein-Barr virus (Likic & Kuzmanic, 2004), cytomegalovirus (Nomura *et al.*, 2005), some cases of *Salmonella* (Arora *et al.*, 2011), 2011), *Brucella* (Young *et al.*, 2000), tuberculosis (Dagaonkar & Udwadia, 2012), malaria (Gupta *et al.*, 2013), *Toxoplasma* (Tuon *et al.*, 2008), and leishmania can lead to Spleenomegaly. It can also be

caused due to Infiltrative disease which includes Gaucher's disease, amyloidosis, and glycogen storage disease, systemic lupus erythematosus, and Felty's syndrome.

- ***Increased Platelet Destruction:***

Platelet destruction results from various immune conditions, including the following:

1. Immune thrombocytopenic purpura (ITP)
2. Thrombotic microangiopathies.
3. Post-transfusion purpura (PTP)
4. Heparin-induced thrombocytopenia (HIT)
5. Disseminated intravascular coagulation (DIC)

The prevalence of ITP is around 100 cases per 1,000,000 person/years, with 50% of cases occurring in the paediatric age group. However it can also be seen as adult or childhood onset. Adult onset is more likely to be chronic and insidious and more common in females than in male, whereas childhood onset has equal gender distribution (Cines & Blanchette, 2002).

Treatment includes use of methylprednisolone, 30mg/kg/day to a maximum of 1g/day and/or IVIG, 1g/kg/day, for 2 to 3 days. The treatment may be combined with platelet transfusions if condition deteriorates or if patient has to undergo surgery. Splenectomy is most promising with a 66% response rate and is indicated in patients in which it relapses and do not respond to treatment with steroids, IVIG, or Rh_o(D). Immune globulins are also known to be associated with side effects.

- ***Platelet Transfusion:***

Diseases like dengue, hemorrhagic fever, Immune thrombocytopenia purpura, Systemic Erthymatosis Lupus are some of the diseases which require platelet transfusion. Platelet transfusion may often be required for patients that have recently undergone chemotherapy. However, unlike other blood components platelets need to be stored at room temperature which limits the shelf life of the product and increase risk of bacterial and fungal growth. According to British committee for standards in haematology, platelet transfusion administered in bleeding disorders is one of the most expensive procedure. Limited centers are available, in the developing world, for platelet separation and safe storage. Therefore the need arises for alternate, safe and inexpensive therapies or new sources of platelet production.

1.5 Use of Herbs in Medicine:

The use of plants in medicine is as old as civilization. Almost in all the ancient civilizations plants have been major part of their medicines. In fact the oldest written evidence of medicinal plant's usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, which is believed to be approximately 5000 years old. It comprises 12 recipes for drug preparation, referring to over 250 various plants, where some of them include alkaloid from plants such as poppy, henbane, and mandrake (Kelly, 2009). Similarly collection of 800 proscriptions, referring to 700 plant species and drugs from plants such as pomegranate, castor, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury have been found in The Ebers Papyrus (Glesinger, 1954;

Tucakov, 1964). Even today many Indian tribals are dependent only upon plants. Their knowledge in treating many of the diseases and healing lethal wounds is vast.

The beginning of the use of medicinal plants in treatment was instinctive (Stojanoski, 1999) as there was inadequate information either regarding the reasons for illnesses or the plant's curative ability. The tribes generally relied on their own or their forefather's experience. Gradually the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered. Thus, the medicinal plant's usage gradually abandoned the empiric framework and became known on explicatory facts. Plants had been the source of treatment and prophylaxis until the advent of iatrochemistry in 16th century (Kelly, 2009). In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection of Hindu sacred verses which led to a system of health care known as Ayurvedic medicine. In the other parts of the world also, medicinal plants are an important element of indigenous medical systems. For example, in the northwestern Amazon, indigenous people used at least 1300 plant species to create drogas do certão or "wildness drugs" (Schultes, 1979). In Southeast Asia, traditional healers use about 6500 different plants to treat malaria, stomach ulcers, syphilis, and other disorders. Nonetheless, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs newsworthy again. In Civilizations across the world, indigenous people discovered and developed the medicinal uses of native plants, however, the foundations of Western medicine were established from the herbal medicine of ancient Greece. This can be traced back to the Greek physician Hippocrates (460-377 BC), who is known as the Father of Medicine. Later on William Withering became the first person in the medical field to scientifically

investigate a folk remedy. His studies (1775-1785) of foxglove, as a treatment for dropsy, set the standard for pharmaceutical chemistry. In the nineteenth century scientists began to purify the active extracts from medicinal plants, wherein breakthrough was achieved by Friedrich Serturner in pharmaceutical chemistry when he isolated morphine from the opium poppy (*Papaver somniferum*) in 1806 (Sertürner, 1805). Similarly, in 1827, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities (Buchner, 1828). It was first synthesized in 1853 which led to the development of aspirin, the most widely used synthetic drug today. Although the direct use of plant extracts decreased in the late nineteenth and early twentieth centuries, medicinal plants still contribute significantly to prescription drugs. It is estimated that 25 % of prescriptions written in the United States contain plant-derived active ingredients which can be close to 50 % if fungal products are included in the list (Fox, 1994). The knowledge and ideas related to the usage of medicinal plants, has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services. The enthnobotany and ubiquitous plants provide a rich resource for natural drug research and development (Cox & Balick, 1994). Though western medicine drifted away from use of plants, 75 % to 90 % of the rural population in other parts of the world still relies on plant products and herbal medicine as their only means of health care (Robinson & Zhang, 2011). Even in present day, China, India, and many countries in Africa and South America have long and ongoing tradition of herbal medicines (Misra, 2013). In fact at many village market places in these countries, medicinal herbs are sold alongside vegetables and other wares. Practitioners of herbal medicine undergo a rigorous and extended training to learn the

names, uses, and preparation of native plants from the local people. In recent years, the plant research and use of traditional medicine information has gained considerable interest (WHO, 2004). Substantial number of industrial and academic researchers have acknowledged the use of plant resources and indigenous rights on the plant resources (Taylor *et al.*, 2001). However, the use of such information has also come under increasing scrutiny. Meanwhile, the need for basic scientific investigations on medicinal plants using indigenous medical systems become imminent (Kong *et al.*, 2003). Today, less than 1 % of the world's tropical forest plants have been tested for pharmaceutical properties, yet at least 25 % of all modern drugs originally came from rainforests (Balick & Mendelsohn, 1992), most of which were first discovered and used by indigenous peoples. A noteworthy step was taken, by the Government of Belize to preserve the valuable knowledge of medicinal plant. A sanctuary, "Terra Nova Forest Reserve" spread across 6000-acre and open to traditional healers, was established and dedicated to survival of medicinal plant. (Balick *et al.*, 1994). List of some of the Drugs/chemicals with their action/ Clinical use and plant uses is given in Table 1.1

Table 1.1: List of Drugs/ Chemicals with their action/clinical use and plant source

D**RUG/CHEMICAL**	**ACTION/CLINICAL USE**	**PLANT SOURCE**
Atropine	Anticholinergic	*Atropa belladonna*
Bergenin	Antitussive	*Ardisia japonica*
Betulinic acid	Anticancerous	*Betula alba*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubefacient	*Cinnamomum camphora*
Cocaine	Local anaesthetic	*Erythroxylum coca*
Codeine	Analgesic, Antitussive	*Papaver somniferum*

Colchicine	Antitumor agent, Anti-gout	*Colchicum autumnale*
L-Dopa	Anti-parkinsonism	*Mucuna sp*
Ephedrine	Sympathomimetic, Antihistamine	*Ephedra sinica*
Gossypol	Male contraceptive	*Gossypium sp*
Menthol	Rubefacient	*Mentha species*
Methyl salicylate	Rubefacient	*Gaultheria procumbens*
Morphine	Analgesic	*Papaver somniferum*
Nicotine	Insecticide	*Nicotiana tabacum*
Papain	**Proteolytic, mucolytic**	***Carica papaya***
Quinine	Antimalarial, antipyretic	*Cinchona ledgeriana*
Reserpine	Antihypertensive, tranquillizer	*Rauvolfia serpentine*
Rutin	Capillary fragility	*Citrus species*
Scopolamine	Sedative	*Datura species*
Silymarin	Antihepatotoxic	*Silybum*
Taxol	Antitumor agent	*Taxus brevifolia*
Vincristine	Antitumor, Antileukemic agent	*Catharanthus roseus*

1.5.1 *Carica papaya*:

Carica papaya, is an evergreen, giant herbaceous tree of the Caricaceae (papaya) family with origin in Central America. It is now grown in tropical areas world-wide for its large, sweet, melon-like fruits. It is usually 2-10m tall and unbranched. However, branching may occur due to injury. All the parts of this plant contain white latex in large or small amounts. Its stem is cylindrical with 10-30cm diameter, hollow with spongy-fibrous tissue and prominent leaf scars. Its root system is extensive; leaves are spirally arranged and clustered near apex of trunk with petiole up to 1m long, hollow, greenish or purplish-green in colour. Leaf lamina is orbicular, 25-75 cm in diameter.

Its leaves are palmate, deeply 7-lobed, glabrous, prominently veined with lobes deeply and broadly toothed.

Flowers of *Carica papaya* are tiny, yellowish white, funnel-shaped, solitary or clustered

Cited from: http://suntrees.co.za/carica-papaya-papaya-tree/

in the leaf axils of the plant. As the plant is dioceous, male and female flowers are separate, however hermaphrodite species are also available. Female flowers are 3-5 cm long with large functional pistil but no stamens and ovoid-shaped ovary; male flowers have long hanging panicles, with 10 stamens in 2 rows, gynoecium is absent except for a

pistillode. Hermaphrodite flowers are larger than male flowers with 5-carpellate ovary but their occurrence depends on the season or age of the tree.

Fruit of *Carica papaya* is large, cylindrical, with fleshy orange pulp with hollow cavity. The skin is thin and yellowish when ripe and green when unripen. Fruits when formed from female flowers are oblong, spherical and pear-shaped while long, obovoid or pyriform when from hermaphrodite flowers. Seeds are numerous, small, black, round and covered with gelatinous aril. Small latex vessels extend throughout the tree and are particularly abundant in the fruit that has reached full size but has not yet begun to ripen.

C. papaya grows satisfactorily in a wide range of areas from the equatorial tropics to temperate latitudes. However, it must be grown in warm, sunny sites sheltered from wind; preferably below 1500m of altitude. Strong winds are detrimental, particularly on soils that cannot make up for large transpiration loss. *C. papaya* is not frost hardy; exposure to frost or cold winds usually results in leaf damage and subsequent death of the tree. Roots are very sensitive to water logging, and even short periods of flooding can kill the plant.

- ***Uses:***

C. papaya is cultivated for its edible ripe fruit. The juice of the fruit is a popular beverage, and its young leaves, shoots, and fruits are cooked as a vegetable. The fruits are a source of flavoring used in candies, jellies and ice creams (Villegas, 1997) and is said to be a rich source of vitamin A, C, and calcium. There are many commercial products derived from different parts of the *C. papaya* plant, the most prominent being papain and chymopapain, which is produced from the latex of the young fruits, stem, and the leaves.

C. papaya leaves have been used in folk medicine for centuries. Shallow cuts on the surface of fully grown but unripe fruits cause a milky sap or latex to ooze that is collected, dried, and termed "crude papain" (Poulter & Caygill, 1985). Papain has many industrial uses, as well as milk-clotting (rennet) and protein-digesting properties. Nearly 80% of American beer is treated with papain, which allows the beer to remain clear upon cooling (Panda, 2017). Papain is most commonly used as commercial meat tenderizers and chewing gums. Cosmetically, papain is used in some toothpastes (Kalyana *et al.*, 2011), shampoos, and facial creams (Chen *et al.*, 2017). Recent studies have shown its beneficial effect as an anti-inflammatory agent (Owoyele *et al.*, 2008), for its wound healing properties (Gurung & Skalko-Basnet, 2009), antitumour as well as immune-modulatory effects (Otsuki *et al.*, 2010) and as an antioxidant (Imaga *et al.*, 2010). A toxicity study (acute, subacute, and chronic toxicity) conducted on Sprague Dawley rats administered with *Carica papaya* leaves juice (CPLJ) of the Sekaki variant revealed has that it is safe for oral consumption (Halim *et al.*, 2011).

Several reports are available for use of *Carica papaya* leaves in increasing blood platelet count in dengue heomrrgeic fever (Ahmad *et al.*, 2011; Kasture *et al.*, 2016; Subenthiran *et al.*, 2013).

Aim of Present Study:

In lieu of the above literature, the primary aim of the study was to scientifically validate the platelet increasing property of *Carica Papaya* Leaf Extract (CPLE) *in vivo*. To reach the goal, studies including designing of a Thrombocytopenia Rat and Mice Model and Toxicity, were carried out. Idiopathic Thrombocytopenia Purpura being a rare but serious and relatively unexplored disease, an additional aim was included to explore the association of few candidate genes with disease in Indian, specifically Gujarat population. This included the study of Single Nucleotide Polymorphism (SNP) of five candidate genes. SNP is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (e.g. > 1%). SNPs are responsible for the differences in Human susceptibility to disease.

Objective of the Study:

- Assessment of Toxicity profile of *Carica papaya* leaf extract (CPLE) in murine model
- Assessment of *invitro* toxicity of CPLE in terms of ROS generation and inflammation in HepG2 Cell line.
- Development of Murine model of thrombocytopenia – pharmacological and Immunological
- Evaluation of Platelet increasing potential of CPLE in Murine Model
- Assessment of Single Nucleotide polymorphism of inflammatory cytokine in pathogenesis of Chronic Immune Thrombocytopenia Purpura patients

1.6 BIBLIOGRAPHY

Ahmad, N., Fazal, H., Ayaz, M., Abbasi, B. H., Mohammad, I., & Fazal, L. (2011). Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pacific Journal of Tropical Biomedicine, 1*(4), 330-333. doi:10.1016/S2221-1691(11)60055-5

Al Ghumlas, A. K., & AbdelGader, A. G. (2003). The liver and the haemeostatic system. *Saudi J Gastroenterol, 9*(2), 59-68.

Araneda, M., Krishnan, V., Hall, K., Kalbfleisch, J., Krishnaswamy, G., & Krishnan, K. (2001). Reactive and clonal thrombocytosis: proinflammatory and hematopoietic cytokines and acute phase proteins. *South Med J, 94*(4), 417-420.

Arora, S., Gupta, N., Kumar, A., & Kaur, I. (2011). Salmonella enteritidis from a case of fever with thrombocytopenia. *Asian Pacific Journal of Tropical Medicine, 4*(4), 328-329.

Balick, M. J., Arvigo, R., & Romero, L. (1994). The development of an ethnobiomedical forest reserve in Belize: its role in the preservation of biological and cultural diversity. *Conservation Biology, 8*(1), 316-317.

Balick, M. J., & Mendelsohn, R. (1992). Assessing the economic value of traditional medicines from tropical rain forests. *Conservation Biology, 6*(1), 128-130.

Bizzozero, G. (1881). Su di un nuovo elemento morfologico del sangue dei mammiferi e della sua importanza nella trombosi e nella coagulazione. *L'Osservatore, 17*(3).

Bizzozero, G. (1883). *Di un Nuovo Elemento morfologico del Sangua e della sua importanza nella Trombosi e nella coagulazione*: Fr. Wallardi.

Boccardo, P., Remuzzi, G., & Galbusera, M. (2004). *Platelet dysfunction in renal failure.* Paper presented at the Seminars in Thrombosis and Hemostasis.

Buchner, A. (1828). Ueber das Rigatellische Fiebermittel und über eine in der Weidenrinde entdeckte alcaloidische Substanz. *Repertorium für die Pharmacie, 29*, 405-420.

Chen, Y.-Y., Lu, Y.-H., Ma, C.-H., Tao, W.-W., Zhu, J.-J., & Zhang, X. (2017). A novel elastic liposome for skin delivery of papain and its application on hypertrophic scar. *Biomedicine & Pharmacotherapy, 87*, 82-91.

Cines, D. B., & Blanchette, V. S. (2002). Immune thrombocytopenic purpura. *N Engl J Med, 2002*(346), 995-1008.

Cox, P. A., & Balick, M. J. (1994). The ethnobotanical approach to drug discovery. *Scientific American, 270*(6), 82.

Dagaonkar, R. S., & Udwadia, Z. F. (2012). Disseminated tuberculosis with immune thrombocytopenic purpura. *Lung India: official organ of Indian Chest Society, 29*(1), 63.

Donne, A. F. (1842). *De l'origine des globules du sang, de leur mode de formatton et de leur fin*.

Ebbe, S. (1976). Biology of megakaryocytes. *Progress in hemostasis and thrombosis, 3*, 211.

Fox, R. (1994). Pharmaceuticals from plants: great potential, few funds. *Lancet, 343*, 1513-1515.

Glesinger, L. (1954). Medicine through centuries. *Zagreb: Zora*, 21-38.

Gupta, N. K., Bansal, S. B., Jain, U. C., & Sahare, K. (2013). Study of thrombocytopenia in patients of malaria. *Tropical parasitology, 3*(1), 58.

Gurung, S., & Skalko-Basnet, N. (2009). Wound healing properties of *Carica papaya* latex: in vivo evaluation in mice burn model. *J Ethnopharmacol, 121*(2), 338-341. doi:10.1016/j.jep.2008.10.030

Halim, S., Abdullah, N., Afzan, A., Rashid, B. A., Jantan, I., & Ismail, Z. (2011). Acute toxicity study of *Carica papaya* leaf extract in Sprague Dawley rats. *Journal of Medicinal Plants Research, 5*(10), 1867-1872.

Hawkey, C. M. (2013). *Comparative mammalian haematology: cellular components and blood coagulation of captive wild animals*: Butterworth-Heinemann.

Hewson, W. (1771). *An experimental inquiry into the properties of the blood*: Cadell.

Imaga, N. A., Gbenle, G. O., Okochi, V. I., Adenekan, S., Duro-Emmanuel, T., Oyeniyi, B., . . . Ekeh, F. C. (2010). Phytochemical and antioxidant nutrient constituents of *Carica papaya* and Parquetina nigrescens extracts. *Scientific Research and Essays, 5*(16), 2201-2205.

Kalyana, P., Shashidhar, A., Meghashyam, B., SreeVidya, K., & Sweta, S. (2011). Stain removal efficacy of a novel dentifrice containing papain and Bromelain extracts–an in vitro study. *International Journal of Dental Hygiene, 9*(3), 229-233.

Kasture, P. N., Nagabhushan, K., & Kumar, A. (2016). A multi-centric, double blind, placebo controlled, randomized, prospective study to evaluate the efficacy and safety of *Carica papaya* leaf extract, as empirical therapy for thrombocytopenia associated with dengue fever. *J Assoc Physicians India, 64*, 15-20.

Kelly, K. (2009). *History of medicine* New York, USA: Facts on file, Inc., An Imprint of Infobase Publisher.

Kong, J. M., Goh, N. K., Chia, L. S., & Chia, T. F. (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharmacol Sin, 24*(1), 7-21.

Kurata, Y., Hayashi, S., Kiyoi, T., Kosugi, S., Kashiwagi, H., Honda, S., & Tomiyama, Y. (2001). Diagnostic value of tests for reticulated platelets, plasma glycocalicin, and thrombopoietin levels for discriminating between hyperdestructive and hypoplastic thrombocytopenia. *American Journal of Clinical Pathology, 115*(5), 656-664.

Kuter, D. J., & Begley, C. G. (2002). Recombinant human thrombopoietin: basic biology and evaluation of clinical studies. *Blood, 100*(10), 3457-3469.

Likic, R., & Kuzmanic, D. (2004). Severe thrombocytopenia as a complication of acute Epstein-Barr virus infection. *Wiener Klinische Wochenschrift, 116*(1), 47-50.

Loll, P. J., Picot, D., & Garavito, R. M. (1995). The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. *Nature Structural & Molecular Biology, 2*(8), 637-643.

Long, M. W., Williams, N., & Ebbe, S. (1982). Immature megakaryocytes in the mouse: physical characteristics, cell cycle status, and in vitro responsiveness to thrombopoietic stimulatory factor. *Blood, 59*(3), 569-575.

Mammen, E. F., Comp, P. C., Gosselin, R., Greenberg, C., Hoots, W. K., Kessler, C. M., . . . Nugent, D. J. (1998). PFA-100 system: a new method for assessment of platelet dysfunction. *Semin Thromb Hemost, 24*(2), 195-202. doi:10.1055/s-2007-995840

Mantadakis, E., Tsalkidis, A., & Chatzimichael, A. (2008). Thrombocytosis in childhood. *Indian Pediatr, 45*(8), 669-677.

Misra, L. (2013). A review on traditional phytomedicinal systems, scientific validations and current popularity as nutraceuticals. *International Journal of Traditional and Natural Medicines, 2*(1), 27-75.

Nomura, K., Matsumoto, Y., Kotoura, Y., Shimizu, D., Kamitsuji, Y., Horiike, S., & Tamiwaki, M. (2005). Thrombocytopenia due to cytomegalovirus infection in an immunocompetent adult. *Hematology, 10*(5), 405-406.

Odell, T., Jackson, C., & Friday, T. (1970). Megakaryocytopoiesis in rats with special reference to polyploidy. *Blood, 35*(6), 775-782.

Otsuki, N., Dang, N. H., Kumagai, E., Kondo, A., Iwata, S., & Morimoto, C. (2010). Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. *J Ethnopharmacol, 127*(3), 760-767. doi:10.1016/j.jep.2009.11.024

Owoyele, B. V., Adebukola, O. M., Funmilayo, A. A., & Soladoye, A. O. (2008). Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. *Inflammopharmacology, 16*(4), 168-173. doi:10.1007/s10787-008-7008-0

Panda, H. (2017). *Carica papaya* : papaya : cultivation, processing, utilizations and applications New Delhi, India: Discovery Publishing House Pvt.Ltd.

Pedersen, A. K., & FitzGerald, G. A. (1985). Cyclooxygenase inhibition, platelet function, and metabolite formation during chronic sulfinpyrazone dosing. *Clinical Pharmacology & Therapeutics, 37*(1), 36-42.

Poulter, N., & Caygill, J. (1985). Production and utilization of papain-a proteolytic enzyme from *Carica papaya* L. *Trop. Sci, 25*, 123-137.

Radley, J., & Haller, C. (1982). The demarcation membrane system of the megakaryocyte: a misnomer? *Blood, 60*(1), 213-219.

Robinson, M. M., & Zhang, X. (2011). The world medicines situation 2011, Traditional medicines: Global situation, issues and challenges. *World Health Organization, Geneva*.

Schultes, R. E. (1979). The Amazonia as a source of new economic plants. *Economic Botany, 33*(3), 259-266.

Schultze, M. (1865). Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes. *Archiv für Mikroskopische Anatomie, 1*(1), 1-42.

Sertürner, F. (1805). "Säure im Opium.". *Journal der Pharmacie, 13*(1), 234f.

Skoda, R. C. (2009). Thrombocytosis. *Hematology Am Soc Hematol Educ Program*, 159-167. doi:10.1182/asheducation-2009.1.159

Stojanoski, N. (1999). Development of health culture in Veles and its region from the past to the end of the 20th century. *Veles: Society of Science and Art*, 13-34.

Subenthiran, S., Choon, T. C., Cheong, K. C., Thayan, R., Teck, M. B., Muniandy, P. K., . . . Ismail, Z. (2013). *Carica papaya* Leaves Juice Significantly Accelerates the Rate of Increase in Platelet Count among Patients with Dengue Fever and Dengue Haemorrhagic Fever. *Evid Based Complement Alternat Med, 2013*, 616737. doi:10.1155/2013/616737

Taylor, J., Rabe, T., McGaw, L., Jäger, A., & Van Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. *Plant Growth regulation, 34*(1), 23-37.

Tefferi, A., Ho, T. C., Ahmann, G. J., Katzmann, J. A., & Greipp, P. R. (1994). Plasma interleukin-6 and C-reactive protein levels in reactive versus clonal thrombocytosis. *Am J Med, 97*(4), 374-378.

Tucakov, J. (1964). *Pharmacognosy. Beograd: Institute for text book issuing in SR. Srbije.*, 11-30.

Tuon, F. F., Higashino, H. R., Amato, V. S., & Nicodemo, A. C. (2008). Acute immune-mediated thrombocytopenic purpura related to *Toxoplasma gondii* infection. *International Journal of Infectious Diseases, 12*(6), 671-672.

Van Leeuwenhoek, A. (1674). Microscopical observations concerning blood, milk, bones, the brain, spittle and cuticula. *Philosoph Trans Roy Soc London, 9*, 121-128.

Villegas, V. (1997). *Carica papaya* L *Edible fruits and nuts* (Vol. 2). Wageningen University, The Netherlands: EWM Verheij, RE Coronel, eds.

Vora, A. J., & Lilleyman, J. S. (1993). Secondary thrombocytosis. *Archives of Disease in Childhood, 68*(1), 88-90.

Weigert, A. L., & Schafer, A. I. (1998). Uremic bleeding: pathogenesis and therapy. *The American Journal of The Medical Sciences, 316*(2), 94-104.

WHO. (2004). WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. Retrieved from http://apps.who.int/iris/handle/10665/43034

Wilson, P. A., McNicol, G. P., & Douglas, A. S. (1968). Effect of fibrinogen degradation products on platelet aggregation. *Journal of Clinical Pathology, 21*(2), 147-153.

Young, E. J., Tarry, A., Genta, R. M., Ayden, N., & Gotuzzo, E. (2000). Thrombocytopenic purpura associated with brucellosis: Report of 2 cases and literature review. *Clinical Infectious Diseases, 31*(4), 904-909.