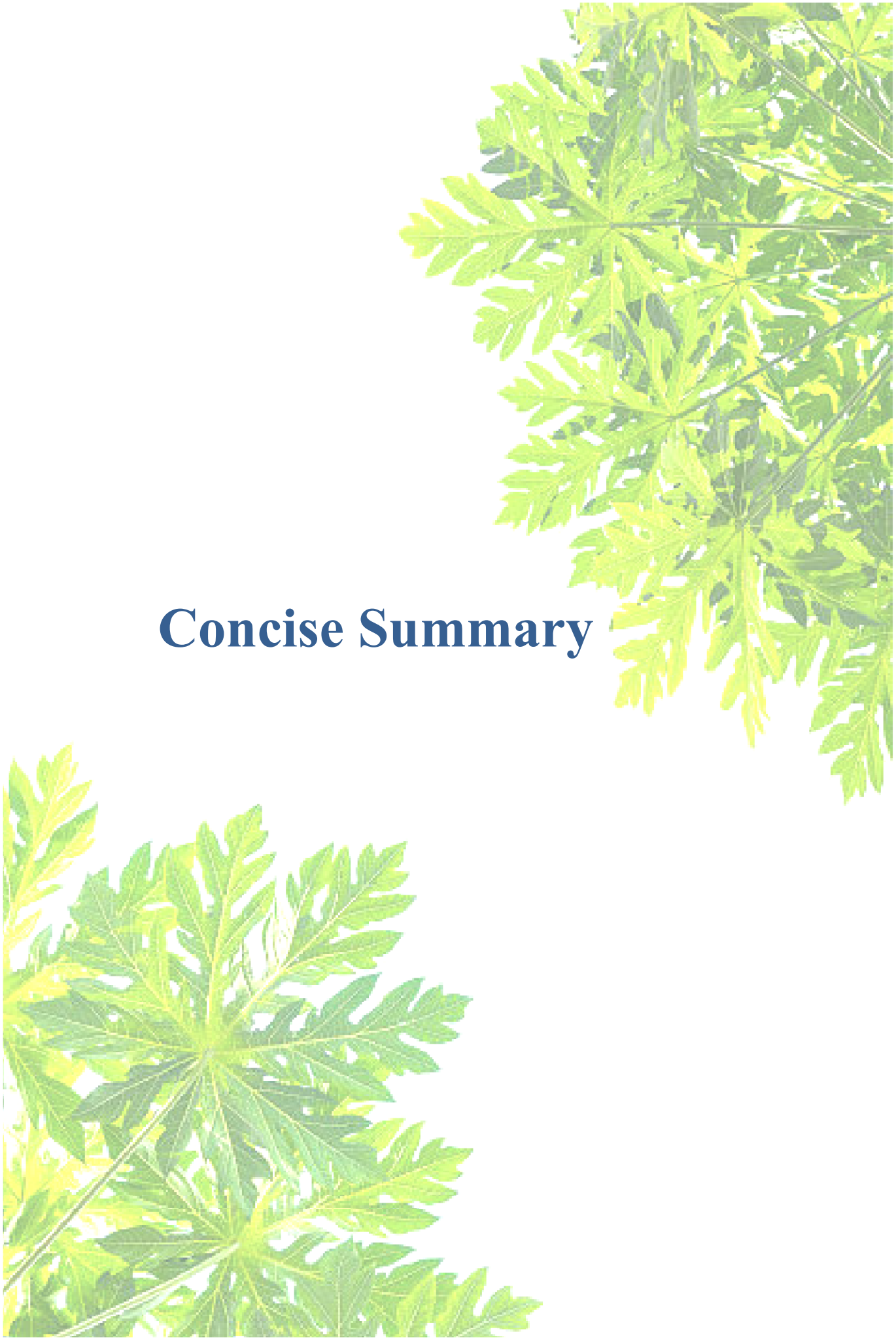

Concise Summary

CONCISE SUMMARY

Platelets are anucleated blood cells that play a central role in hemostasis and thrombosis and contribute to diverse processes, including atherosclerosis, angiogenesis, vascular restenosis, wound healing, immune responses and inflammation. Low blood platelet count can have adverse effects and may become fatal if untreated. The condition of low platelet count is clincally known as Thrombocytopenia. Thrombocytopenia is defined as a platelet count less than 150,000/mL. It is considered to be mild when the platelet count is between 70,000 and 150,000/mL, and severe if less than 20,000/mL. Most individuals are asymptomatic if the platelet count is 50,000/mL or greater. Diseases such as dengue, Idiopathic Thrombocytopenic Purpura (ITP), Thrombotic Thrombocytopenia (TTP) and Systemic Lupus Erythematosus (SLE) result in a low thrombocyte count in the blood. But in case of potentially life-threatening complications platelet transfusions are required to prevent the of severe thrombocytopenia which is seen in a variety of medical settings including cancer therapy, trauma, and sepsis. Compounding these platelet supply challenges are issues with individual units, including variations in platelet number and functionality, room temperature storage requirements that increase the risk of bacterial contamination, up to 5-day expiration dates resulting in discarded units that create wastage, and platelet short half-lives of 1.5 to 3 days following infusion. The generation of an efficient, non-donor-dependent system for platelet production to supplement the donor-derived pool could address many of these concerns. Thereby the need arises for easily available yet potent compound to increase platelets in these conditions. The use of herbal medicines in treatment diseases and various conditions is age old. Many plants and

herbs or their active molecules derived from them are part inseparable part modern medicine. *Carica papaya* is one such plant. *C. papaya* is common plant found in the tropics. The whole plant from roots to leaves even the plant sap has potential role in treating many disease. The plant is exploited by the tribal of Eastern and middle east countries. Some of the modern day studies have highlighted *C. papaya* role in increasing platelet count in Dengue, also some of primary studies have done in murine model showing platelet increasing activity of C. papaya. Hence the present study was under taken to evaluate the toxicity of *C. papaya* leaf extract in murine models and cell lines, Phamacological and immune model of Thrombocytopenia, Evaluation of Platelet increasing activity of *C. papaya* leaf extract. The study also projects to observe the SNPs involved in Immune Thrombocytopenia Purpura patients and to evaluate the associated SNPS with the disease in Gujarat population.

Evaluation of *Carica papaya* leaf extract toxicity in Murine Model.

This study was aimed to determine the *invivo* toxicity profile of CPLE in murine models. To achieve these, 30 healthy rats were acclimatized for period of 7 days. In Acute study animals were given single dose of 1000 mg/kg BW for 24 hrs and blood profile, serum biochemistry and histology profile was assessed. In a parallel studies the animals were treated with 10 mg/kg BW, 100 mg/kg BW and 1000 mg/kg BW, similar to Acute studies Blood and serum profile were assessed and histology was performed. The results of the did not show any significant changes in blood profile in acute as well as chronic toxicity studies, Also histology of liver, kidney, spleen and ovary did not show any sign of tissue insult, and were similar to control animals. However we found dose and time dependent

increase in blood platelets as per our hypothesis in all the groups subjected to 100 mg/kg BW CPLE the difference was most significant effect in lower dose compare to other with respect to control.

Inflammation and Cytotoxicity assessment of CPLE in HepG2 cell line.

Cyotoxicity studies were carried out with CPLE in HEPG2 Cell line. For the first part of the study cells were treated with 5ug/ml to 640 ug/ml dose CPLE and MTT assay was performed to determine the cell viability after treatment. There was no significant decrease in viability was observed. To study the intracellular oxidative damage due to CPLE cells were treated with 50 ug/ml and 100ug/ml CPLE. H2O2 was kept as positive control. Post treatment cells were treated with DCF-DA and subjected to FACS to determine the ROS levels, there was no significant difference in the cells intensity in treated groups (50 and 100 ug/ml), while H2O2 group showed increased cell population with high DCF-DA intensity. Also IL-6 levels in supernatant were analyzed to determine the inflammation, there was increase in the IL-6 levels, but this increase can be correlated with increased thrombopoetin production in hepatocytes as IL-6 promotes thrombopoetin expression. Thus there was cytotoxicity associated with the CPLE.

Effect of platelet reducing drugs in rat: An attempt for pharmacological thrombocytopenic model making.

This study was carried out to make pharmacological and antibody mediated model of thrombocytopenia. Mice were subjected Vancomycin at dose of 5 mg/kg Bw and 10 mg/kg BW as vancomycin is known to cause thrombocytopenia in many patients. However we did not find any significant change in blood platelet level. Similarly mice were treated with Hydroxyurea alone at dose of 15 mg/kg BW and 25 mg/kg BW did not give any significant decrease in number of platelet. Hydroxyurea was also tried in association with Aspirin as both are known to decrease blood platelets. Yet there was no significant change in platelet number however in high dose (25 mg hydroxyurea and 15 mg Aspirin) there was significant decrease h but the levels returned back to normal and the decrease in count cannot be called as thrombocytopenia clinically. Anagrelide the drug know to be used in essential thrombocythemia to reduce platelet was utillzed at 5 ug and 10 ug/kgBw in mice, this drug also did not decrease blood platelet numbers thus we could establish pharmacological model of thrombocytopenia. Finally we tried with Antibody mediated platelet destruction which showed positive results. Animals were treated with 4ug antibody per mice. The results showed immediate and significant lowering of platelets (in 6 hrs). This immune model was utilized in successive studies.

Platelet increasing potential of *Carica papaya* leaf extract in Murine model.

To asses this mice were given CPLE at dose of 10mg/kg BW, 100 mg/kg BW, 1000 mg/kg BW, for 28 days, mice were assessed for blood platelet count. Results revealed that there was significant increase in blood platelet in all the doses. Now to answer whether CPLE inhibited destruction of platelets or it increased new platelet count a study was conducted using Flowcytometry. Her mice were divided in 4 groups control, only CPLE 100 mg/kg BW, CPLE in antibody induced thrombocytopenia and Thrombocytopenic animals alone. From our previous studies we found the 4ug antibody was inducing thrombocytopenia in mice. The platelets were initially labelled with Biotin (which labels all the platelets at time), than mice were treated with antibody and CPLE as per groups made. Blood was withdrawn; PRP was isolated and was subjected CD41 platelet selection. This CD41 population was checked for Streptavidin positive population in all the groups. Results revealed that there was decrease in streptavidin population as well as CD41 positive population, Whereas in CPLE alone treatment there was new increased CD41 population and streptavidin population remained same. In Thrombocytopenic mice also CPLE increased blood platelet count that can be reaveled by increased CD41 population and no streptavidin population. In confirmatory studies same groups were treated with Thiazole orange (TO) without streptavidin, as TO labels reticulocyte population. Results reveled to there was new population formed that can be seen increased peak of TO in Flow cytometry in CPLE treated groups with respect to control. This was even shown in thrombocytopenic animals.

Single Nucleotide Polymorphism of selected candidate genes in pathogenesis of Immune Thrombocytopenia Purpura in Population from Gujarat.

Immune Thrombocytopenic Purpura (ITP) is an autoimmune disease characterized by the presence of antibodies against self platelets. The etiology of ITP remains unclear, but it is generally accepted that both environmental and genetic factors and probably also a synergistic relationship between these factors, play important roles in the development of the disease. Here we have investigated the association of TNF α (-308G/A), TNF β (+252A/G), IL4 (-590C/T), IL4 intron 3 VNTR and IL10 (-592C/A; -1082G/A) polymorphisms with Immune thrombocytopenia Purpura in West Indian Population. For this blood was taken from confirmed ITP patients, DNA was extracted from it. The extracted DNA was utilized for PCR-RFLP analysis. Association of above mentioned SNPs were checked in patients with respect to 103 controls. Results showed that TT polymorphism was more associated in IL-590 polymorphism. R2R2 polymorphism dominance was seen in patient population however we did not find R1R1 homzygous. Similar results were observed in IL10-1082 AA polymorphism where there was significant association and in TNF A patient population was also associated with the disease. TNF B polymorphism did not show any significance association.