Journal of Ethnopharmacology 275 (2021) 114074

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Platelet enhancement by *Carica papaya* L. leaf fractions in cyclophosphamide induced thrombocytopenic rats is due to elevated expression of CD110 receptor on megakaryocytes

C. Nandini [a], SubbaRao V. Madhunapantula [b,1,**], Venugopal R. Bovilla [b], Mohammad Ali [a], K. Mruthunjaya [c], Manjula N. Santhepete [a,*], K. Jayashree [d]

[a] Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India
[b] Centre of Excellence in Molecular Biology and Regenerative Medicine (CEMR), JSS Medical College, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India
[c] Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India
[d] Department of Pathology, JSS Medical College and Hospital, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India

ARTICLE INFO

Keywords:
Thrombocytopenia
Thrombopoietin
CD110/cMpl receptor
Carica papaya leaf
Megakaryocytes

ABSTRACT

Ethnopharmacological relevance: Carica papaya leaf juice/decoction has been in use in folk medicine in Srilanka, Malaysia and in few parts of India for enhancing the platelet counts in dengue. In Siddha medicine, a traditional form of medicine in India, papaya leaf juice has been used for increasing the platelet counts. Papaya leaf has been reported to enhance blood volume in ancient Ayurveda books in India. *Carica papaya* leaf is well known for its platelet enhancement activity. Although many preclinical and clinical studies have demonstrated the ability of papaya leaf juice for platelet enhancement, but the underlying mechanisms are still unclear.

Aim of the study: The study is aimed at identifying the key ingredients of papaya leaf extract and elucidate the mechanism (s) of action of the identified potent component in mitigating thrombocytopenia (Thp).

Materials and methods: C. papaya leaf juice was subjected for sequential fractionation to identify the anti-thrombocytopenic phytochemicals. *In vivo*, stable thrombocytopenia was induced by subcutaneous injection of 70 mg/kg cyclophosphamide (Cyp). After induction, rats were treated with 200 and 400 mg/kg body weight papaya leaf juice and with identified fractions for 14 days. Serum thrombopoietin level was estimated using ELISA. CD110/cMpl, a receptor for thrombopoietin on platelets was measured by western blotting.

Results: Administration of cyclophosphamide for 6 days induced thrombocytopenia ($210.4 \pm 14.2 \times 10^3$ cells/μL) in rats. Treating thrombocytopenic rats with papaya leaf juice and butanol fraction for 14 days significantly increased the platelet count to 1073.50 ± 29.6 and $1189.80 \pm 36.5 \times 10^3$ cells/μL, respectively. *C.papaya* extracts normalized the elevated bleeding and clotting time and decreased oxidative markers by increasing endogenous antioxidants. A marginal increase in the serum thrombopoietin (TPO) level was observed in Cyp treated group compared to normal and treatment groups. Low expression of CD110/cMpl receptor found in Cyp treated group was enhanced by *C. papaya* extracts (CPJ) and CPJ-BT. Furthermore, examination of the morphology of bone marrow megakaryocytes, histopathology of liver and kidneys revealed the ability of CPJ and fractions in mitigating Cyp-induced thrombocytopenia in rats.

Conclusion: C. papaya leaf juice enhances the platelet count in chemotherapy-induced thrombocytopenia by increasing the expression of CD110 receptor on the megakaryocytes. Hence, activating CD110 receptor might be a viable strategy to increase the platelet production in individuals suffering from thrombocytopenia.

* Corresponding author. Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, 570015, Karnataka, India.
** Corresponding author. Center of Excellence in Molecular Biology and Regenerative Medicine (CEMR) (DST-FIST Sponsored Center), JSS Medical College, JSS Academy of Higher Education & Research, Mysuru, 570015, Karnataka, India.
E-mail addresses: mvsstsubbarao@jssuni.edu.in (S.V. Madhunapantula), snmanjula@jssuni.edu.in (M.N. Santhepete), jayashreekrishnamurthy62@gmail.com (K. Jayashree).
[1] Leader, Special Interest Group in Cancer Biology and Cancer Stem Cells (SIG-CBCSC), JSS Academy of Higher Education & Research, Mysuru - 570,015, Karnataka, India.

https://doi.org/10.1016/j.jep.2021.114074
Received 27 October 2019; Received in revised form 23 January 2021; Accepted 23 March 2021
Available online 5 April 2021
0378-8741/

1. Introduction

Platelets are nucleated or non-nucleated blood cells produced in bone marrow, which play an important role in the maintenance of hemostasis (MedCrave, 2015). Low platelet count (100×10^3 cells/μL), a condition known as Thrombocytopenia (Thp), and/or abnormal platelet function causes spontaneous bleeding (Uhl et al., 2017). Thrombocytopenia is a condition indicated by hematological abnormalities that include internal/external bleeding. The cause of Thp may be due to decrease in platelet production, increased platelet destruction or sequestration of platelet in the spleen (Visentin and Liu, 2007). Low platelet count is one of the secondary complications associated with many diseases such as, leukemia, liver cirrhosis, and viral infections like dengue, Ebola, and parasitic infection such as malaria (Azeredo et al., 2015), (McElroy, 2015), (Gupta et al., 2013). Decrease in platelet number might also be due to intake of drugs such as heparin, chloramphenicol, and anticancer agents (Nandini et al., 2016). A very serious problem of Thp is spontaneous bleeding as a resultant of which organ failure, stroke, and finally death might occur. The severity of bleeding can be decreased by increasing the platelet count (Carson and Stanworth, 2017).Thrombocytopenia is one of the harmful side effects of chemotherapy (Psaila et al., 2012) Chemotherapy drugs might either cause a direct toxic effect on bone marrow cells or decrease the production of platelet (Weycker et al., 2019). Recent studies have also shown the displacement of normal bone marrow cells by excessively growing cancer cells, especially in case of leukemia or lymphomas (Liebman, 2014). In the presence of cancer cells, the bone marrow fails to produce platelets effectively. Thrombocytopenia creates a number of problems in case of cancer patients. Whereas at platelet count <10,000 cells/μL, spontaneous bleeding occurs (Aravind et al., 2008). the surgical procedures are often complicated by bleeding if the platelet counts <50,000/μL. At platelet counts <100,000/μL, chemotherapy, and radiation therapy are administered with caution for fear of causes of thrombocytopenia and increasing the risk of bleeding (David J. Kuter, 2015). Reduction in the use of anti-cancer agents, or frequent platelet transfusions are the only treatment options available to balance the platelet counts in chemotherapy induced thrombocytopenia. However, key mechanisms leading to chemotherapy-induced thrombocytopenia are still unclear (Hassan and Waller, 2015).

Chemotherapy-induced thrombocytopenia is a common complication affecting 20 to 60 percent of patients globally (Weycker et al., 2019). Among various chemotherapeutic agents, alkylating pharmacological molecules such as cyclophosphamide are known to cause thrombocytopenia in rats (Vadhan-Raj, 2009). Although, many animal models representing thrombocytopenia are available (Chow et al., 2010), alcohol liver disease (ALD) -induced thrombocytopenia (Mutlag et al., 2018)and drug-induced Thp(Reilly and McKenzie, 2002) are widely reported. Among all the animal models drug-induced Thp, especially chemotherapy-drug induced Thp models are well established and extensively studied. However, the patho-physiology and the cause of Thp is different in these models (Neschadim and Branch, 2016), (Mitchell et al., 2016). Immune thrombocytopenia (ITP) is another animal model reported in the literature. However, induction of immune thrombocytopenia using knockout mice is cost effective and need super-specialty care. ALD-induced Thp is not stable and may be reversible (Laffi et al., 2007). Hence, in this research article, an attempt was made to induce thrombocytopenia by administering chemotherapy drug cyclophosphamide. In general, thrombocytopenia is induced by chemotherapy drugs such as carboplatin (McElroy et al., 2015), busulfan (Zunjar et al., 2016), cyclophosphamide (Akhter et al., 2014) and others whereas Cyclophosphamide induced thrombocytopenia is one of the stable (Nie et al., 2009) and general model for studying chemotherapy-induced thrombocytopenia preclinically (Eltantawy et al., 2018), (Anjum et al., 2017). Cyclophosphamide induces thrombocytopenia in mice and rats by suppressing the maturation of bone marrow megakaryocytes (Zhou et al., 2005). Existing methods for treating thrombocytopenia are nonspecific and are known to cause systemic toxicity (Terrell et al., 2020; Samson et al., 2019). Reducing the dose of chemotherapy or intensity of radiation therapy may control the toxicity, but fail to reduce thrombocytopenia. Platelet transfusion is a readily available treatment to control thrombocytopenia (ten Berg et al., 2011). Another method of treating thrombocytopenia is to induce the production of platelets by administering Interleukin 11 (IL-11) and recombinant thrombopoietin by injection (Bhatia et al., 2007). Although recombinant thrombopoietin reduces the chemotherapy-related thrombocytopenia in early clinical trials, subsequent development was halted due to "antibody formation against endogenous thrombopoietin" (Newland, 2009). Eltrombopag is one of the marketed oral drug available for immune thrombocytopenic purpura (ITP) patients to normalize the platelet counts. However, due to its side effects such as hepatic dysfunction and reticulin fibrosis of bone marrow, its usage is restricted (Rice, 2009), (Merli et al., 2015). Hence, to date, no specific drug for enhancing the platelet count in thrombocytopenia is available. Cyclophosphamide, a commonly used pharmacological agent to induce thrombocytopenia, is a synthetic alkylating agent chemically related to the nitrogen mustards with antineoplastic and immunosuppressive effects (Ahlmann and Hempel, 2016). Recent studies have shown that cyclophosphamide can affect megakaryocyte progenitor cells thereby increase the production of immature cells, which leads to decreased platelet count (David J. Kuter, 2015). In addition, cyclophosphamide can cause liver sinusoidal damage and decrease thrombopoietin production leading to thrombocytopenia (Shokrzadeh et al., 2014). Active cyclophosphamide metabolites, aldophosphamide and phosphoramide mustard, which are produced in liver, bind to DNA and inhibit its' replication and initiate cell death (Nie et al., 2009). The renal cleavage of inactive circulating metabolites leads to the generation of toxic byproducts such as acrolein and cause cystitis (Cox and Abel, 1979). Cardiac toxicity is the most severe dose-limited toxicity of cyclophosphamide. In addition, cyclophosphamide has been reported to induce chromosome aberration in bone marrow and liver cells (Moore et al., 1995).

Carica papaya, commonly known as Papaya, is a tropical tree belonging to the Caricaceae family (Gunde and Amnerkar, 2016). Parts of papaya plants especially fruits, seeds, leaf, latex are being used in many studies to modulate immune responses. In addition, phytochemical extracts of papaya have been reported to exhibit antimalarial and antiulcer properties (Gupta et al., 2013).Extract of papaya seed has been used to treat bleeding piles and enlarged liver and spleen. Likewise, papaya stem bark was used in treating hemolysis and jaundice (Krishna et al., 2008). Different parts of papaya plant are reported to contain a variety of chemicals that include, Protein, fat, fiber, carbohydrates, minerals: calcium, iron, vitamin C, thiamine, rivoflavin, niacin, and carotene, anino acid, citric acid and malic acids (green fruits), volatile compounds: benzylisothiocynate, cis and trans 2, 6-dimethyl-3,6 epoxy-7 octen-2-ol, alkaloids in fruits, papaya seed containing the Fatty acids, crude protein, crude fiber, papaya oil, carpaine, caricin, glucotropacolin, and an enzyme myrosin. Whereas, Root and leaves contain Carposides and an enzyme myrossin and Alkaloids carpain, pseudocarpain, dehydrocarpaine I and II, choline, vitamine C and E, carposide respectively. Papain, chemopapain, peptidase A and B, lysozymes were reported in the latex.(Gunde and Amnerkar, 2016), (Vij and Prashar, 2015) Zunjar et al., (2015), reported the presence of carbohydrates, amino acids, saponin, glycosides, iridoids, flavonoids, phenolics, and alkaloids in papaya leaf dried extracts (Zunjar et al., 2015). *Carica papaya* leaf extract is in wide usage in tropical countries including India, Malaysia, Srilanka, Pakistan and Bangladesh for enhancing the platelet counts in dengue fever (Jayasinghe et al., 2017; Subenthiran et al., 2013; Zunjar et al., 2016). In addition, traditional medical practices such as Siddha Medicine of Tamil Nadu region in India prescribes papaya leaf juice to treat dengue fever as well as to enhance blood quality (Manohar, 2013) Tribals of the Bihar in India are using papaya leaves as an antidote for viral infection (Kumar et al., 1998). Preclinical studies have

demonstrated that suspension of *Carica papaya* leaves in palm oil helps in reducing dengue in Swiss albino mice (Sathasivam et al., 2009). Mechanistically, papaya leaf juice enhanced the platelet count in dengue condition by decreasing the expression of NS1 proteins in DENV-infected THP-1 cells in rats (Sharma and Mishra, 2014). Patil et al. have reported potent anti-thrombocytopenic activity of *Carica papaya* leaf extract using the cyclophosphamide-induced thrombocytopenic model of Wistar rats (Patil et al., 2013). Tahir et al., Al (2014), stated that papaya leaf juice prevents the fall of platelet counts in myelo-suppressed Swiss albino mice induced by carboplatin administration (Tahir et al., 2014). Zunar et al., 2016, carried out an extensive study on identifying the active component "carpain" in papaya leaf juice which enhanced the platelet count in busulfan induced thrombocytopenia in rats (Zunjar et al., 2016). Otsuki et al., (2010), studied the cytotoxic effect of *Carica papaya* leaf extracts on PBMC using ELISA, microarray, and RT-PCR ((Otsuki et al., 2010).

Clinically, papaya leaf juice capsules assisted in optimizing the blood platelet count in dengue patients (Arya and Agarwal, 2014; Siddique et al., 2014; Yunita et al., 2012). Subenthiran et al., 2013, reported elevated expression of arachidonate 12-lipoxygenase (ALOX12; EC1.13.11.31) and platelet activating receptor (PTAFR) genes in the dengue patients treated with papaya leaves (Subenthiran et al., 2013). Results of preclinical and clinical studies suggest that the papaya leaf extract contain potent pharmacological agents that help in the treatment and prevention of cancer, allergic disorders, and acts as an immune-adjuvant for vaccine therapy (Otsuki et al., 2010). Although several studies have reported, the efficacy of papaya leaf juice for enhancing platelet count and the mechanism(s) responsible for this property are not fully clear. Therefore, in this preclinical study, an attempt was made to determine the effect of papaya extract on thrombopoietin (TPO) and thrombopoietin receptor CD110/cMpl (Myeloproliferative leukemia protein), as well as on the levels of platelet count. Since platelet generation is controlled by thrombopoietin (TPO), which acts through its receptor cMpl, assessing its expression and activity is highly significant (Bacon et al., 1995). In addition to determining the efficacy of papaya leaf juice for mitigating thrombocytopenia, an effort was also made to fractionate papaya leaf juice for identifying key phytochemicals. Results of this study revealed that papaya leaf juice contain phenolic compounds that play vital role in enhancing the platelet count through promoting the expression of cMpl receptor on platelets while regulating the serum TPO level.

2. Materials and methods

2.1. Chemicals and reagents

Cyclophosphamide injection I.P (Endoxan-N 1 gm) was procured from Zydus cadila healthcare (Biogen) India, and dissolved in 50 ml of sterile water to induce thrombocytopenia. Ethylenediamine tetra acetic acid (EDTA) and Acid citrate dextrose (ACD) vacutainers were purchased from microSD India Private Ltd. Bengaluru, Karnataka, India. Aspartate transaminase (AST), alanyl transaminase (ALT) and bilirubin kits were from Spinreact, Girona, Spain. Thrombopoietin ELISA kit was purchased from E-Labscience Houston, Texas, USA. cMpl antibody was purchased from Sigma Aldrich, St Louis, USA. HPLC standards of all Phenolics acids, p-Coumaric acid (Cat.No:C9008-5G, 98.0%), Chlorogenic acid (Cat. No: C3878-250 MG, 95%), Sinapic acid (Cat. No: D7927-1G, 98%), 3,4-Dimethoxybenzoic acid (Cat.No:D131806-100G, 99%), 3,4-Dihydroxybenzoic acid (Cat. No:37,580-250G-F, 97%), Vanillic acid (Cat. No:94,770-10G, 97.0%), 4-Hydroxybenzoic acid (Cat. No: W398608, 99%), Syringic acid (Cat. No: S6881-5G, 95%), 3,4,5-Trimethoxybenzoic acid (Cat.No: T69000-5G, 99%), 3,4,5-Trimethoxycinnamic acid (Cat. No: T70408-25G, 97%), Caffeic acid (Cat. No: C0625-2G, 98%), 2,5-Dihydroxybenzoic acid (Cat. No:149,357-10G, 98%), Benzoic acid (Cat. No:242,381-25G, 99.5%), 4-Hydroxy-3 methoxy cinnamic acid (Cat. No:H1634-5G, 98%), Trans-Cinnamic acid (Cat.No: C80857-5G, 99%), Dimethyl caffeic acid (D133809-25G, 99%), Caffeic acid (C0625-2G, 98.0%), Ferulic acid (H1634, 98%) Quercetin (Cat. No:117-39-5G-99%) and kaempferol (Cat. No: K0133-10 MG-98%) were procured from Sigma Aldrich, USA.

2.2. Preparation of Carica papaya leaf juice spray dried powder (CPJ)

Carica papaya leaves were collected in autumn season in the month of September 2015 from locality of Mysore district, JSS Ayurveda College, Mysuru. The plants were grown without supplementing any fertilizers and pesticides. Papaya leaves (from male plant) were identified and authenticated by taxonomist Dr. Naga Nandini, Professor, Dept. of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India. A specimen of the collected plant was stored in Dept. of Botany, Manasa Gangotri, University of Mysore, Mysuru, Karnataka, India (The herbarium voucher no:3247 in Supplemental Fig. 2A and 2B). The plant name was confirmed by further checking with http://WWW.theplantlist.org. First, fresh green leaves were washed with running water for several times to remove debris. Next, leaves were ground with blender without adding water and squeezed using clean cotton cloth to prepare *Carica papaya* leaf juice (CPJ). Leaf juice was spray dried using a spray drier & stored in air-tight container.

2.3. Sequential fractionation of Carica papaya leaf juice spray dried powder

Hundred grams of *Carica papaya* leaf juice spray dried power was suspended in 200 ml of water to make the suspension in a separatory funnel. To that equal amount of petroleum ether (CPJ-PE) was added and shake the mixture well and allowed it to stand until two separate clear layer were formed. Than collect the upper petroleum ether layer and repeated the process for three times. Then, ethyl acetate (CPJ-EA) was added to the aqueous layer and extract in the same way. Finally, n-butanol was added to the aqueous layer and repeat the extraction for 3 times and collected n-butanoic fraction (CPJ-BT) by leaving behind the residual of aqueous fraction. Evaporated the solvents from all the extracts using rotavapor and subsequently dried for a very brief period using spray drying to remove the solvent. Then, the sample was dried using lyophilization, weighed and calculated the % of yield.

2.4. Analysis of CPJ fractions using RP-HPLC

Qualitative analysis of *C. papaya* leaf juice spray dried powder & fractions were carried out using Shimadzu Prominence-i LC-2030C RP-HPLC system (Boligon and Athayde, 2014). Experimentally, samples (1 mg/mL) were dissolved in the mobile phase consisting of water: methanol: acetic acid at 80:15:5 ratio (Subba Rao and Muralikrishna, 2002; Seal, 2016). The column (C18, 4.6 × 250 mm) was first equilibrated with mobile phase and base line adjusted. A 20 μL sample (from a stock of 250 μg/mL) was loaded using an auto-sampler (SIL-20ACHT) and the elution monitored for a period of 30min at 250 nm and 280 nm using UV detector (SPD-20A/20AV dual-wavelength absorbance detectors). The flow rate was set at 1.0 ml/min. Comparing the retention time of the peaks with standard phenolic acids retention time, the eluted samples were identified. (Supplemental Tables 1; Supplemental Fig. 1 and 1A to 1D). Standards used for analysis include, Caffeic acid, Gallic acid, Ferulic acid, Syringic acid, Coumaric acid, Coumaric acid, Protocatechuic acid, Quercetin, Sinapic acid, Veratric acid, Kaempferol, o-methyl sinapic acid, Benzoic acid, o-methyl syringic acid, Dimethyl caffeic acid, Chlorogenic acid, Vanillic acid.

2.5. Phytochemical analysis of CPJ and its fractions using liquid chromatography - mass spectrometry

Phytochemical analysis of *C. papaya* leaf juice spray dried powdered

and its fractions were carried out using ALS1260 infinity G1329B standard auto sampler system coupled to a high-resolution accurate mass quadrupole time of flight (QToF) mass spectrometer operating with dual electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) modes. The column (3 × 30 cm Agilent 1260 Infinity Thermostatted Column - C18) housed in SL G1316B TCC (Thermostatted Column Compartment) was first equilibrated using mobile phase (Water containing 0.1% formic acid and Acetonitrile in the ratio of 95:5 in gradient manner) with the flow rate of 0.3 ml/min. Samples 1 mg/ml concentration were dissolved in 70% methanol (CPJ-PE,CPJ-EA and CPJ-BT, CPJ dissolved in distilled water) and 20 μL injected. MS analysis was carried out by both positive and negative ionization mode.{Bystrom, 2008 #43} The mass range of m/z 100–1700 Da, at the scan rate of 3 cycles/sec, was selected with the following acquisition parameters: capillary voltage 2500 V, nebulizer pressure 40 psi, drying gas flow 10 L/min, gas temperature 325 °C, fragmenting voltage 175 V, skimmer voltage 65 V. Chromatogram and mass spectra were analyzed by comparing with standard phenolic acids and flavonoids elution and fragmentation profile as well as the mass spectral details of phenolic acids were cross verified by comparing the already reported spectra in previous reports (Bystrom et al., 2008; Koley et al., 2020) (Table 3, Supplemental Fig. 3A and 3B; Supplemental Figures 4 to 7).

2.6. Anti-thrombocytopenic potential of CPJ and its fractions against cyclophosphamide-induced thrombocytopenia in rats

Male *Sprague Dawley* (SD) rats weighing 180–200 g were used in this study. Animals were procured from a registered breeder (*In vivo* Bioscience, Bengaluru, Karnataka, India). The Institutional Animal Ethics Committee (IAEC), JSS College of Pharmacy, Mysuru, Karnataka, India, approved the protocol (Approval number: 147/2014). The animal experiment was carried out as per the guidelines described by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Department of Animal Husbandry and Dairying, Ministry of Agriculture and Farmers Welfare, Government of India. Animals were allowed to acclimatize for 7 days at laboratory condition (55% humidity, 22–25 °C temperature and lighting sequence of 12 h light, 12 h dark phases). All animals were fed standard rat pellet and water ad libitum.

2.7. Acute toxicity of CPJ and its fractions

Acute oral toxicity test was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline for testing of chemicals: Acute oral toxicity – Fixed dose procedure (OECD Test Guideline 420). Female SD rats were dosed with CPJ, CPJPE, CPJEA and CPJBT in a stepwise procedure using fixed doses of 5, 50, 300 and 2000 mg/kg. Starting with 5 mg/kg body weight, the test material was administered orally at once. The rat was then observed for toxic effect for the next 30 min, followed by hourly for 4 h for the first 24 h. If no signs of toxic effects such as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma or mortality were observed within 24 h, then dosed another rat with the next dose (50 mg/kg BW) and a similar procedure was carried out for all the remaining doses. Rats were monitored and observed once daily for the next 13 days. Based on the acute toxicity data, 2 doses viz., 200 mg/kg and 400 mg/kg body weight, were selected for efficacy study (Halim et al., 2011). SD rats were divided in to 11 groups with 8 animals in each group as detailed in Table 1.

2.8. Induction of thrombocytopenia (Thp)

Injecting three alternative subcutaneous injections of cyclophosphamide (CYP; 70 mg/kg body weight; subcutaneous) induced stable thrombocytopenia in rats. The injected animals were left for 4 days to develop stable thrombocytopenia as confirmed by (a) bleeding and clotting time; (b) decreased platelet count; (c) increased prothrombin time (PT) (Fig. 1) (Bordoloi et al., 2016), (Kristiana et al., 2013).

Table 1
Grouping of Sprague Dawley rats. Grouping of animals or treatment schedule, normal animals received water as vehicle for 24 days, control (Thp) group received 70 mg/kg B.wt of 3 subcutaneous injection of cyclophosphamide and Hydrocortisone served as a standard treatment for 14 days. After induction, animals were treated with *C. papaya* leaf extract (CPJ) and identified Fractions CPJ-PE, CPJ-BT, CPJ-EA at different doses for 14 days. platelet count was measured on day 10 after induction to confirm thrombocytopenia. treatment with CPJ and the Fractions was initiated on Day 11 and continued till Day 24. BT, CT, PT and Platelet count were estimated on Day 18 by collecting the blood. Animals were sacrificed on Day 24.

Group Number	Group Name	Treatment and Route of Administration	Duration (in days)
1	Normal	Vehicle	24 days
2	Thrombocytopenia Control*	3 alternative subcutaneous injections of 70 mg/kg cyclophosphamide.	10 days of induction, 14 days without Treatment
3	Hydrocortisone*	0.5 mg/kg ip.	14 days after induction
4	CPJ-1	200 mg/kg body weight *C. papaya* leaf juice extract PO.	14 days after induction
5	CPJ-2	400 mg/kg body weight *C. papaya* leaf juice extract PO.	14 days after induction
6	CPJ-PE1	200 mg/kg body weight petroleum ether fraction of *C. papaya* leaf juice PO.	14 days after induction
7	CPJ-PE2	400 mg/kg body weight petroleum ether fraction of *C. papaya* leaf juice PO.	14 days after induction
8	CPJ-EA1	200 mg/kg body weight ethyl acetate fraction of *C. papaya* leaf juice PO.	14 days after induction
9	CPJ-EA2	400 mg/kg body weight ethyl acetate fraction of *C. papaya* leaf juice PO.	14 days after induction
10	CPJ-BT1	200 mg/kg body weight butanol fraction of *C. papaya* leaf juice PO.	14 days after induction
11	CPJ-BT2	400 mg/kg body weight butanol fraction of *C. papaya* leaf juice PO.	14 days after induction

2.9. Bleeding time (BT) and clotting time (CT) determination

Bleeding time and clotting time were performed at 10^{th}, 18^{th} and 24^{th} day of treatment. Bleeding time was determined by duke's method and Clotting time was determined according to the method described by Garcia et al. (2016) (Garcia-Manzano et al., 2001),. The normal bleeding time of the rat was 1.5–3.0 min and clotting time was 3.5–5 min respectively.

2.10. Platelet count determination

Platelet count was determined by collecting 0.5 ml of blood from tail tip of rats (from all the groups) on 10th, 18th and 24th day into EDTA K2-anticoagulent tube. Platelet count was determined by using automated hematological analyzer (Celtac α, from NIHON KOHDEN, JAPAN). The normal blood platelet count of the Sprague Dawley rat was 900–1000 × 10^3 cells/μl.

2.11. Prothrombin time (PT) analysis

Out of 2.0 ml of blood collected by retro orbital method (from all rats), 1.0 mL was used for PT (Prothrombin time) test. Remaining 1.0 mL

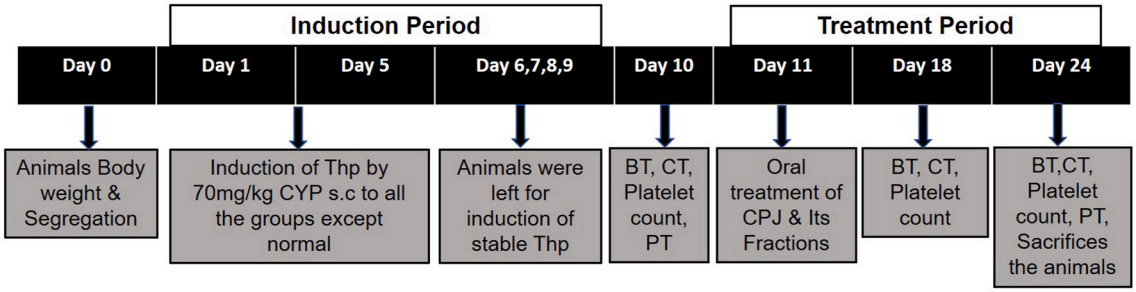

Fig. 1. Schematic representation of the animal experiment procedure: In order to determine the efficacy of *Carica papaya* juice (CPJ) and the Fractions CPJ-PE, CPJ-BT, CPJ-EA, a cyclophosphamide-induced thrombocytopenia (Thp) model was used as detailed in materials and methods. Thp was induced by 3 alternative injection of 70 mg/kg body weight cyclophosphamide subcutaneously. The CYP injected animals were left for 4 days and Bleeding Time (BT), Clotting Time (CT) and Prothrombin Time (PT); and platelet count were measured on Day 10. The treatment with CPJ and the Fractions was initiated on Day 11 and continued till Day 24. BT, CT, PT and Platelet count were estimated on Day 18 and Day 24 by collecting the blood. Animals were sacrificed on Day 24.

blood was used to separate serum for biochemical analysis. Blood was collected in to sodium-citrate vacutainer tubes, mixed well and PT determined using fully automated coagulation analyzer Operon XL-1000i.

2.12. Biochemical analysis of serum

After collection of blood, it was allowed to clot and the tubes were centrifuged at 3000 rpm for 7 min. The separated serum was used to estimate AST, ALT and Total Bilirubin level by Merck semi auto analyzer using spin-react commercially available kits.

2.13. Preparation of platelet-rich plasma (PRP)

5.0 mL blood was collected into acid citrate dextrose (ACD) tubes from all the rats and mixed well. Collected blood was centrifuged at 214xg for 5min (as a soft spin). The upper layer and middle layer were collected in to a new centrifuge tube and spun at 595xg for 17 min. Two third of supernatant, which contain platelet poor plasma, was discarded. Then 1/3rd of PPP (platelet poor plasma) was mixed with Pellet to obtain platelet rich plasma (PRP). PRP is stable for 6 h. To collect protein lysates for Western blotting analysis, PRP was centrifuged at 1792 g for 5min, and the pellet was washed with PBS (2 times). Next, lysis buffer (1x RIPA buffer containing 20 mM Tris-HCl (pH 7.5) 150 mM NaCl, 1 mM Na2EDTA 1 mM EGTA 1% NP-40 1% sodium deoxycholate 2.5 mM sodium pyrophosphate 1 mM β-glycerophosphate 1 mM Na_3VO_4 1 μg/mL leupeptin. 1. At pH 7.5) supplemented with protease inhibitor cocktail was added and generated lysates stored at −80 °C (Dhurat and Sukesh, 2014), (Etulain et al., 2018).

2.14. Estimation of protein

Total protein content in the platelet lysate was determined using Pierce BCA kit from Thermo Fischer Scientific (Brown et al., 1989). A calibration graph was prepared by incubating increasing concentration of 25, 125, 250, 500, 750, 1000 and 1500 μg/mL bovine serum albumin (BSA, 10.0 μL) with 200 μL BCA reaction mixture containing of 50 parts of reagent A (made up of 0.8% sodium bicarbonate, 4% bicinchoninic acid and 0.16% sodium tartrate in 0.1 M sodium hydroxide) and1part of reagent B (made up of 4% cupric sulfate) at 37 °C for 30.0 min. The developed color was measured at 562 nm using a multi-mode plate reader (PerkinElmer, Germany). Similarly suitably diluted test samples were also processed and concentration determined using the calibration curve (Jose et al., 2018).

2.15. Serum thrombopoietin analysis by ELISA

TPO level in the serum was measured using commercially available ELISA kit (Rat TPO immunoassay kit from E-Lab Science Technology, Houston, Texas, USA) The assay was performed according to the manufacturer's instructions. In brief, 100 μL (samples and standard 31.25, 62.5, 125, 250, 500,1000 and 2000 pg/mL concentrations) aliquot was added in to pre-coated wells and incubated for 90 min at 37 °C, then 100 μL of 1X biotinylated detection antibody was added to each well and incubated for an hour at 37 °C. After decanting the solution, the wells were washed thrice using 350 μL wash buffer; added 100 μL HRP conjugate and incubated for 30min at 37 °C. The wells were washed 5 times for 2 min each. 90 μL substrate was added and incubated for 15 min at 37 °C in dark. Finally 50 μL stop solution was added to each well and OD was read at 450 nm. Each sample was analyzed in triplicates and results expressed as Mean ± SEM.

2.16. cMpl receptor expression analysis by Western blot

cMpl expression was measured using Western blotting as detailed: First, aliquots of 2×10^7 platelets were lysed with RIPA buffer and boiled for 10 min. Protein content estimated using BCA method, and SDS electrophoresis was performed on 7.5% polyacrylamide gel (25 mA at RT for 90min). Proteins from gel were transferred to PVDF membrane and detected using a rat polyclonal antibody (anti cMpl-TpoR from Sigma Aldrich, St Louis, USA) at 1 μg/mL in TBST. The membrane was washed with TBST for 3 times (5 min each) and 10 ml secondary anti-rabbit HRP antibody (Sigma Aldrich, St Louis, USA) added. After 1 h, the membrane was washed with TBST and levels of cMpl expression detected using enhanced chemiluminescence (ECL) reagent (GE Healthcare, UK). The gel was stripped at 50 °C for 30 min and re-probed with a mouse anti CD41 antibody (SZ22 clone) to measure the expression of fibrinogen receptor on platelets (Vianello et al., 2014). Beta-actin was used as a control for protein loading.

2.17. Examination of bone marrow megakaryocytes morphology

After sacrificing, bone marrow was collected from femur (from all the rats), and a thin smear was prepared on a glass slide. The bone marrow smears were stained with Leishman stain and examined under the microscope using 100x oil immersion. Changes in the number and morphology of megakaryocytes in different groups were observed and photomicrographs were captured using a camera (Vinayakamurthy et al., 2017), (Dameshek and Miller, 1946).

2.18. Determination of endogenous antioxidants

The liver and kidney were isolated from all the rats and perfused with 0.9% cold physiological saline to remove all red blood cells. Then the organs were suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) cut into small pieces, and homogenized using a homogenizer

(Remi RQ-127A/D homogenizer, 8 × 275 length and 100 ml stirring capacity with AC/DC 1/8 HP motor). The homogenate was centrifuged at 11200 g for 20 min at 4 °C. The supernatant was used for the estimation of endogenous Superoxide dismutase (SOD), Catalase and reduced glutathione (GSH) levels. SOD was determined by the method of Mishra and Fridovich et al., 1972 *(Misra and Fridovich, 1972)*. Catalase and GSH were estimated by the method given by Aebi, H. 1984 (Aebi, 1984), and Ellman et al., 1959 (Ellman, 1959), Lipid peroxidation (MDA content) was estimated by the method of Ohkawa and Ohishi respectively (Ohkawa et al., 1979). Protein content in the tissue was determined using biuret method as detailed in a commercially available kit from SPINREACT (SANTESTEVEDEBAS(GI)SPAIN; http://www.spinreact.com/files/Inserts/MD/BIOQUIMICA/MDBSIS30_PROT_TOT_2017.pdf).

2.19. Histopathology

Histopathological evaluation of liver and kidney sections was carried out by first rapidly perfusing the tissues followed by fixing with10% formalin for 24 h. Sections were prepared and stained with hematoxylin and eosin (H&E) for microscopic observation. The sections were examined under the light microscope for histopathological changes of liver sinusoids and kidney cells. A pathologist examined the stained sections and provided the report.

2.20. Statistical analysis

All the values were expressed as Mean +SEM. The data of bleeding- and clotting time, platelet count and prothrombin time were analyzed by Two way ANOVA followed by Bonferroni's post hoc comparison test. Serum biochemical markers, endogenous antioxidant markers were analyzed by One-way ANOVA followed by Tukey's multiple comparison test using Graph pad Prism version 6. "P" value < 0.05 was considered significant.

3. Results

3.1. Yield of C. papaya leaf juice powder and the extracted extracts

To identify the potent anti-thrombocytopenic activity of the Fractions of *Carica papaya* leaf juice spray dried powder was successively fractionated with petroleum ether, ethyl acetate and butanol; and the anti-thrombocytopenic activity was determined using the animal model. *C. papaya* leaf juice powder yield was 72% w/w of leaves. Among the three Fractions generated from papaya leaf juice powder, extraction with petroleum ether (CPJ-PE) produced 28% w/w of yield; while two other Fractions CPJ-EA and CPJ-BT yielded 10% w/w and 18.9% w/w of dry powder respectively. Analysis of solubility properties of Fractions demonstrated 100% solubility of CPJ in water. However, CPJ-PE, CPJ-EA and CPJ-BT exhibited 100% solubility only in organic solvents such as methanol.

3.2. Analysis of CPJ and its fractions using RP-HPLC

In order to determine the phenolic acid composition of CPJ and CPJ Fractions, RP-HPLC was used and the data are represented in Table 2. RP-HPLC is one of the most widely used analytical methods to determine the composition of plant Fractions (Boligon and Athayde, 2014). Analysis of Fractions on C18 column provides key information about the constituent compounds (Kumar, 2017). Therefore, in this study, CPJ and the Fractions CPJ-PE, CPJ-EA and CPJ-BT were analyzed on C18 column as detailed in materials and methods. Analysis of the data showed the presence of 4-hydroxy, 3-methoxy cinnamic acid (HMCA, commonly known as Ferulic acid), 3,4,5-trihydroxy benzoic acid (THBA, commonly known as Gallic acid) and 3,4,5-trihydroxy cinnamic acid (THCA) in the ethyl acetate fraction (Table 2, Supplemental Table 1 and Supplemental Fig. 1 and 1A,-1D). However, phenolic acid rich CPJ-PE, CPJ had O-methylated benzoic acids 3,4-dimethoxy benzoic acid (Veratric acid), 3,4,5-trimethoxy benzoic acid (O-methyl syringic acid), benzoic acid and cinnamic acid derivatives caffeic acid and ferulic acid (Table 2, Supplemental Fig. 1A-1D). Unlike these Fractions, the CPJ-BT had protocatechuic acid, dimethyl caffeic acid, tetrahydroxy flavone and syringic acid/gallic acid.

Table 2
Tentative identification of Phenolic acids and Flavonoids of Carica papaya leaf juice extract and Fractions.
Tentative phytochemical composition of CPJ and its Fractions was determined using RP-HPLC. Compounds were identified by comparing the masses of sample peaks with known standard peaks. Benzoic acid, caffeic acid, chlorogenic acid, coumaric acid gallic acid, veratric acid, Sinapic acid, ferulic acid and dimethyl caffeic acids were used as a standard. Analysis of the MS data showed the presence of Benzoic acid, o-methyl syringic acid, Gallic acid, Ferulic acid and Veratric acid in CPJ (a). The extract that had low antioxidant activity ie., CPJ-PE had Syringic acid and cinnamic acid (b). The fraction CPJ-EA found to contain Ferulic acid, Gallic acid and cinnamic acid (c). The butanol fraction CPJ-BT had Gallic acid, Kaempferol, Dimethyl caffeic acid and Protocatechuic acid (d).

Sl no	Extract/Fractions	Observed Rt	Identified compound
1	Carica papaya leaf juice (CPJ)	3.354	Benzoic acid
		3.471	3,4,5-trimethoxy benzoic acid (o-methyl syringic acid)
		2.801	Caffeic acid/Syringic acid/Gallic acid
		2.963	4-hydroxy 3-methoxy cinnamic acid (Ferulic acid)
		3.095	3,4-dimethoxy benzoic acid (Veratric acid)
2	CPJ-PE	2.801/ 2.815	Caffeic acid/Syringic acid/Gallic acid
		3.471	3,4,5-trimethoxy benzoic acid (o-methyl syringic acid)
		3.246	3,4,5-trihydroxy cinnamic acid
3	CPJ-EA	2.963	4-hydroxy 3-methoxy cinnamic acid (Ferulic acid)
		2.805	3,4,5-trihydroxy benzoic acid (Gallic acid)
		3.246	3,4,5-trihydroxy cinnamic acid
4	CPJ-BT	2.801	Caffeic acid/Syringic acid/Gallic acid
		3.1	3, 5, 7, 4′-tetrahydroxyflavone (Kaempferol)
		3.587	3,4-dimethoxy cinnamic acid (Dimethyl caffeic acid)
		3.013	3,4-dihydroxy benzoic acid (Protocatechuic acid)

3.3. Further identification of phytochemicals of C. papaya leaf juice spray dried powder and its fractions using LCMS

Even though RP-HPLC is widely used to identify phenolic constituents of plant extracts, it is not a fool-proof confirmation. Hence, *C. papaya* Fractions were further characterized using LC-MS. Analysis of the data showed the presence of various types of phenolic acids and flavonoids, derivatives (Table 3, Supplemental Table 2; Supplemental Fig. 3A and 3B and Figs. 4–7). LC-MS analysis of CPJ showed the presence of Quercetin, trans cinnamic acid, 4 hydroxy benzoic acid, Benzoic acid, kaempferol, syringic acid and carpaine. In addition, Chlorogenic acid and carpaine were also found in CPJ (Table 3 and Supplemental Figure 4). The petroleum ether extract CPJ-PE consisted of Chlorogenic acid and carpaine (Table 3 and Supplemental Figure 5). The CPJ-EA was rich in a variety of antioxidants such as 4 hydroxy benzoic acid, Sinapic acid, kaempferol, 2,5 dihydroxy benzoic acid, 3,4,5 tri methoxy cinnamic acid and carpaine (Table 3 and Supplemental Figure 6). Likewise, the CPJ-BT was also found to be rich in Benzoic acid, vanillic acid, syringic acid, P-coumaric acid, Trans cinnamic acid, 4 hydroxy benzoic acid and carpaine. (Table 3 and Supplemental Figure 7).

Table 3
Tentative identification of phytochemicals present in CPJ and its Fractions using UPLC-qTOF/MS.
Phytochemicals present in the papaya leaf juice was tentatively identified by UPLC method by comparing the mass spectra with Standards Mass spectra (Supplemental Fig. 3A and 3B; Supplemental Table 2). as detailed in methods section.

Sl. No.	Extracts	Identified compounds	m/z of features $(M-H)^+$ $(M-H)^-$	Retention time in min	Molecular Formula
1	**CPJ**	Quercetin	302.12	1.12	$C_{15}H_{10}O_7$
2		4-Hydroxy Benzoic acid	138.12	1.26	$C_7H_6O_3$
3		Benzoic acid	120.16	1.54	$C_7H_6O_2$
4		Trans-Cinnamic acid	146.18	1.74	$C_9H_8O_2$
5		Kaempferol	287.13	1.99	$C_{15}H_{10}O_6$
6		Carpaine	479.16	2.99	$C_{28}H_{50}N_2O_4$
7		Unidentified	273.14	1.07	
8		Syringic acid	197.79	1.33	$C_9H_{10}O_5$
9	**CPJ-PE**	Carpaine	479.93	4.47	$C_{28}H_{50}N_2O_4$
10		Chlorogenic acid	353.47	4.97	$C_{16}H_{18}O_9$
11		Unidentified	522.90	5.16	–
12	**CPJ-EA**	4-Hydroxy Benzoic acid	137.17	1.34	$C_7H_6O_3$
13		Sinapic Acid	224.24	1.78	$C_{15}H_{10}O_6$
14		Un identified Peak	–	2.25	–
15		Carpaine	479.49	3.03	$C_{28}H_{50}N_2O_4$
16		Kaempferol	287.19	3.13	$C_{15}H_{10}O_6$
17		2,5 Dihydro Benzoic acid	152.04	1.35	$C_7H_6O_4$
18		Unidentified Peak	–	2.96	–
19		3,4,5 Trimethoxy Cinnamic acid	238.11	3.69	$C_{12}H_{14}O_5$
20	**CPJ-BT**	Benzoic acid	122.18	1.26	$C_7H_6O_2$
21		Vanillic acid	166.22	1.70	$C_8H_8O_4$
22		Un identified Peak	–	1.94	
23		Carpaine	479.55	3.01	$C_{28}H_{50}N_2O_4$
24		Syringic acid	196.27	3.44	$C_9H_{10}O_5$
25		Trans-Cinnamic acid	146.06	1.02	$C_9H_8O_2$
26		4-Hydroxy Benzoic acid	137.05	1.26	$C_7H_6O_3$
27		P-Coumaric acid	164.14	1.70	$C_9H_8O_3$
28		Kaempferol	286.25	3.20	$C_{15}H_{10}O_6$

3.4. Acute toxicity analysis of CPJ and fractions on sparge Dawley rats

Acute toxicity study was conducted for *C. papaya* leaf juice and the Fractions CPJ-PE, CPJ-EA and CPJ-BT on sparge dawley nonpregnant and nulliparous female rats weighing about 180 gm. The study was conducted according to the OECD guildline 423, by implementing a stepwise fixed dose (50, 300 and 2000 mg/kg Body weight) protocol. No mortality was observed during 14 days period in any of these experimental groups. No changes were also observed either in food and water intake, relative weight of internal organs. However, a slight increase in haemoglobin and red blood cell count was observed in CPJ treated group when compared to control group. Furthermore, no changes were observed in skin and fur, eyes and mucus membrane, behavior pattern, salivation, diarrhea, sleep and coma. Based on the acute toxicity data, 1/5th (400 mg/kg body weight), 1/10th (200 mg/kg body weight), and 1/20th (100 mg/kg body weight) of the highest dose was selected for the efficacy study of the extract and Fractions (Ismail et al., 2014).

3.5. Efficacy of CPJ and fractions on clotting cascade

Bleeding and clotting time are the two main parameters that vary significantly in Thrombocytopenia (Bashawri and Ahmed, 2007). Due to cyclophosphamide toxicity, bleeding and clotting time were increased in Thp control group compared to normal animals.

3.5.1. Bleeding time

The Normal bleeding time (1.5–2.5 min) was increased in thrombocytopenia to 4.0 ± 0.183min at day-8 (Fig. 2A). Due to this increase, a spontaneous nose bleeding was observed in the animals. Treatment with papaya leaf juice and its Fractions significantly decreased the bleeding time beginning from day 15. CPJ and CPJ-BT showed better reduction in bleeding time compared to other groups. For instance, the bleeding time was reduced from 4.0 ± 0.13 in Thp control to a level similar to normal animals viz., 2.0 ± 0.17 in CPJ (400 mg/kg body weight) and 2.0 ± 0.56 in CPJ-BT (400 mg/kg body weight) at day 18 (Fig. 2A).

3.5.2. Clotting time

Hemostasis, a process that stops the blood loss or bleeding, occurs as a result of coagulation pathway (Mann et al., 2009). Intrinsic and extrinsic signaling mechanisms, coagulation factors and various proteins influencing the formation of platelet plug regulates hemostasis pathway (Gale, 2011). Clotting time is one of the main parameters considered while assessing hemostasis (Hoffman, 2003). Platelets play an important role in the formation of platelet plug to initiate the clotting cascade. Due to low platelet count and/or abnormal platelet function clotting cascade is interrupted in Thp condition leading to prolonged clotting time (Park et al., 2010). Thrombocytopenia is characterized by a prolonged clotting time (Kamal et al., 2007). In the normal group, clotting time was found to be 3.2 ± 0.21 min, but in thrombocytopenia condition, clotting time increased to 6.3 ± 0.18 min. After the treatment with plant Fractions for 24days, clotting time was normalized in all the groups (Fig. 2B).

3.5.3. Prothrombin time

Another factor indicating the hemostasis process, was prolonged due to thrombocytopenia in rats (19.57 ± 0.39 s in Cyclophosphamide administered thrombocytopenic rats versus 12.60 ± 0.15 s in normal rat). Administration of CPJ decreased prothrombin time to 13.10 ± 0.17 s and 12.50 ± 0.23 s respectively at 200 mg/kg and 400 mg/kg dosage (Fig. 2C). Similarly, oral administration of 400 mg/kg body weight CPJ-PE, CPJ-EA and CPJ-BT also reduced prothrombin time to 13.17 ± 0.17,12.75 ± 0.18 and 12.80 ± 0.14 respectively (Fig. 2C).

3.6. Oral administration of CPJ and its fractions improved platelet count in cyclophosphamide-induced thrombocytopenia

Sub-cutaneous administration of cyclophosphamide-induced thrombocytopenia at 10th day was evidenced by a significant decrease in platelet count from 925.30 ± 18.6 × 10^3 cells/μl to 210.4 ± 14.2 × 10^3 cells/μl (Fig. 3). Nose bleeding was observed after the induction of thrombocytopenia in rats. Oral treatment with CPJ leaf juice and Fractions at different doses increased the platelet count in a dose dependent and time dependent manner (Fig. 3). At 18th day CPJ-1 & CPJ-2, CPJ-BT1 and CPJBT2 showed significant improvement in platelet count ($P < 0.001$). The extent of improvement was even better than the positive control Hydrocortisone (Fig. 3).

3.7. Cyclophosphamide induced AST, ALT and total bilirubin were reduced by CPJ and fractions

Cyclophosphamide is a potent inducer of thrombocytopenia (Aster and Bougie, 2007). In order to determine whether cyclophosphamide induced serum markers were reduced by the administration of CPJ and the Fractions isolated from CPJ, blood from control and experimental animals was collected and the levels of AST, ALT and total bilirubin were measured in serum. There was an increase in serum marker level in ALT (77.56 ± 2.2 U/L), AST (155.91 ± 10.76) of what group, compared to normal group ALT (43.7 ± 0.75) and AST (111.41 ± 0.90). After the

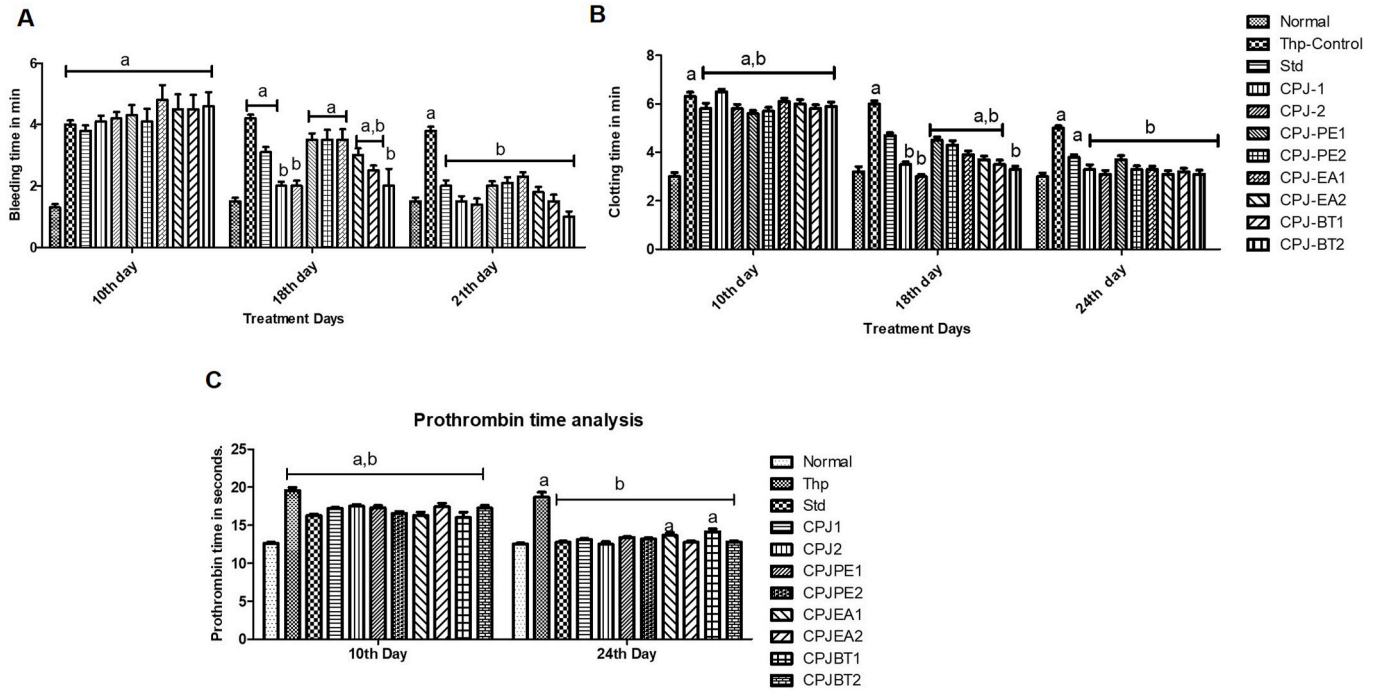

Fig. 2. Effect of *C. papaya* leaf juice and Fractionson Clotting cascade
Bleeding time, clotting time and Prothrombin time (PT) was tested using the blood collected from rats administered with cyclophosphamide (Thp), hydrocortisone (Std), CPJ and CPJ Fractions. Analysis of the data showed elevated BT, CTand PT in Thp rats compared to control untreated ones. Administration of hydrocortisone or the CPJ Fractionsand Fractions reduced the bleeding, clotting and prothrombin time significantly at 24th day. All the values are expressed as Mean ± SEM (n = 8). Data was analyzed using Two way ANOVA with Bonferroni's post hoc test for multiple comparison. $^{a}P<0.05$ compared to normal and $^{b}P < 0.05$ compared to disease control (Thp).

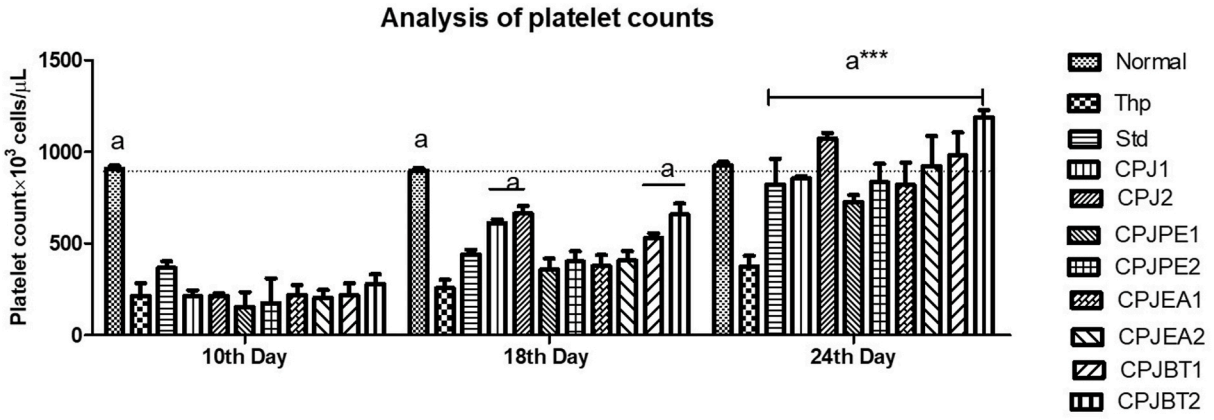

Fig. 3. *C. papaya* leaf juice and the Fractions increased the number of platelets in thrombocytopenic rats: Thrombocytopenia is characterized by a significant decrease in the number of platelets (Left panel). Administration of CPJ or CPJ Fractions increased the platelet count in a time dependent manner. At day 24, the platelet count is equal to normal animals indicating full recovery upon administration of CPJ or CPJ Fractions. All the values are expressed as Mean ± SEM, (n = 8), Data was analyzed using two-way ANOVA with Bonferroni post-hoc test for multiple comparison. $^{a}P<0.001$is compared to normal group with different days of treatment.

treatment with *C. papaya* leaf extract and Fractions for 14 days the level of AST & ALT were restored to normal healthy animals. 400 mg/kg body weight CPJ and CPJBT exhibited better restoration compared to other extracts. For instance, the CPJ (400 mg/kg body weight) administration reduced the ALT and AST levels to 46.7 ± 2.8 U/L and 121.0 ± 17.6 U/L respectively (Fig. 4). The effects produced by CPJ and its Fractions were similar to the standard compound, hydrocortisone (Fig. 4).

Since cyclophosphamide is known for its hepatotoxicity, it is predicted that the levels of bilirubin might be much higher in rats administered with this compound (Snyder et al., 1993). Elevated bilirubin is also responsible for platelet destruction and thrombocytopenic condition (NaveenKumar et al., 2015). As predicted, total bilirubin level was increased to 0.84 ± 0.06 gm/dl in the thrombocytopenic rats (compared to Normal group (0.23 ± 0.02 gm/dl). Elevated total bilirubin was reduced to normal levels by the administration of *C. papaya* leaf juice and Fractions.

3.8. CPJ and CPJ-Fractions reduced cyclophosphamide-triggered liver malondialdehyde (MDA) by augmenting anti-oxidant enzymes

Induction of lipid peroxidation is one of the characteristic features of cyclophosphamide (Ray et al., 2011). Rats administered with

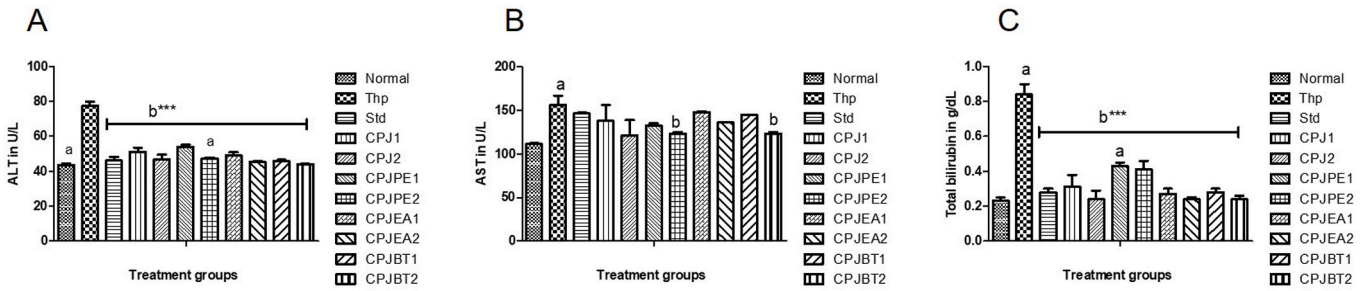

Fig. 4. CPJ and CPJ Fractions reduced the over expressed ALT, AST and total bilirubin content in thrombocytopenic rats: Thrombocytopenic rats exhibited elevated serum ALT, AST and total bilirubin, primarily due to liver damage. Administration of CPJ and CPJ Fractions reduced the levels of ALT, AST and total bilirubin to the levels that were observed in normal healthy rats. All the values were expressed as Mean ± SEM (n = 8), Data was analyzed by One-way ANOVA with Tukey's post-hoc test for multiple comparison. [a]P<0.001is compared to Normal group, [b]P < 0.001 compare to Thp. A) ALT, B) AST and C) Total bilirubin.

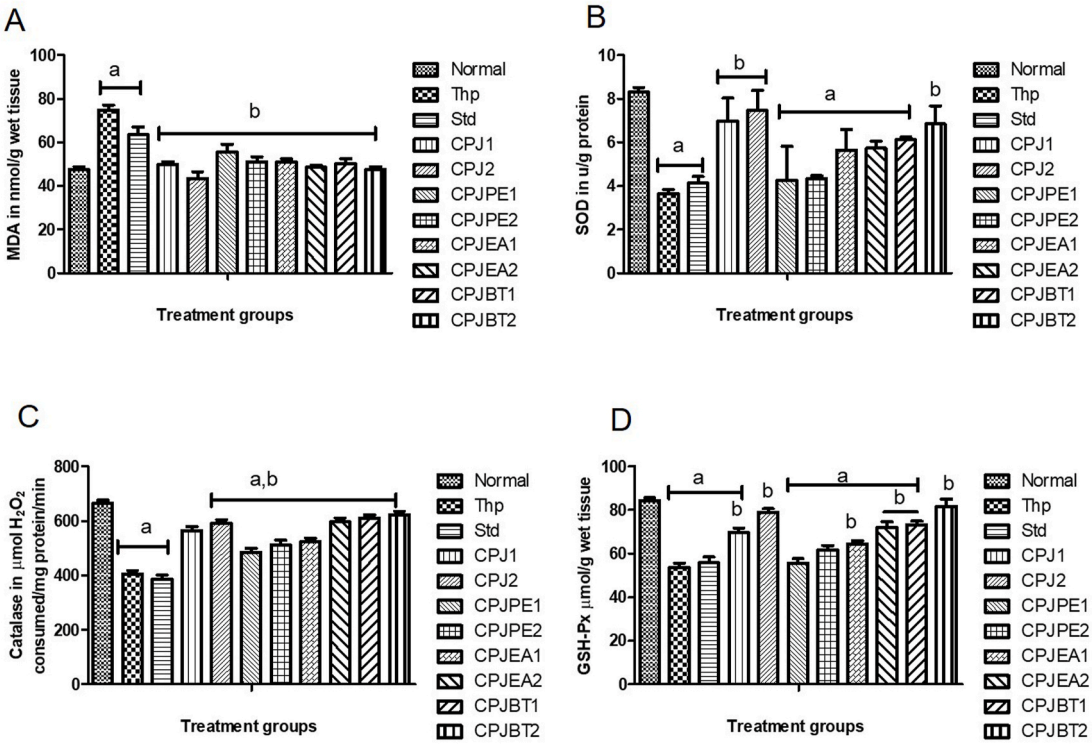

Fig. 5. Effect of *C. papaya* Leaf juice and Fractions on endogenous antioxidants and antioxidant Enzymes: Increased oxidative stress due to cyclophosphamide administration is one of the most devastating factors responsible for cellular and organ damage in rats. Administration of Cyclophosphamide increased cellular MDA level, which was reduced to the levels similar to normal control (not correct) (A). Similarly, cellular super oxide dismutase (SOD), catalase (Cat) and glutathione levels were also increased due to treatment with to have decrease (sentence not correct). All the values were expressed as Mean ± SEM (n = 8), Data was analyzed by One-way ANOVA with Tukey's post-hoc test for multiple comparison. [a]P<0.001is compared to normal group, [b]P < 0.001 compare to Thp and [c]P < 0.001 compare to standard.

cyclophosphamide exhibited elevated ROS (represented by high MDA) levels thereby causing cellular destruction in liver (Fig. 5A). In general, cellular ROS is controlled by the upregulation of defense mechanisms that includes nonenzymatic antioxidants such as glutathione, uric acid, bilirubin, vitamins C and E; and enzymatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Pratico et al., 1992). SOD catalyzes dismutation of the superoxide anion into H_2O_2, while GSH-Px and CAT detoxify H_2O_2 and convert lipid hydroperoxides into nontoxic alcohols (Balahoroğlu et al., 2008). Excess H_2O_2 causes the activation of compliment factor C3 and subsequent binding of Cb-9 complex (on platelet surface) leading to the destruction of platelets in thrombocytopenia condition (Hamad et al., 2010). Therefore, promoting endogenous antioxidants can scavenge the H_2O_2 radicals and prevent the destruction of platelets. Hence, in this study, first the level of lipid peroxidation product MDA (a marker of oxidative stress) was measured in rats treated with cyclophosphamide. Next, the effect of administering CPJ or the Fractions CPJ-PE, CPJ-BT and CPJ-EA on liver MDA level as well as the antioxidant enzymes was assessed. CPJ and the Fractions exhibited reduced MDA levels that are similar to the ones in control normal rats (Fig. 5A). Increased ROS level was also represented by a significantly lower endogenous antioxidant enzymes SOD (Fig. 5B), Catalase (Fig. 5C) and the antioxidant, GSH (Fig. 5D). Administration of CPJ and its Fractions at different doses restored the cellular antioxidant enzymes and GSH (Fig. 5).

3.9. Elevated serum thrombopoietin (TPO) was reduced by CPJ and its fractions treatment

Thrombopoietin is one of the key cytokines responsible for Megakaryopoiesis, a process responsible for the production of platelets. TPO

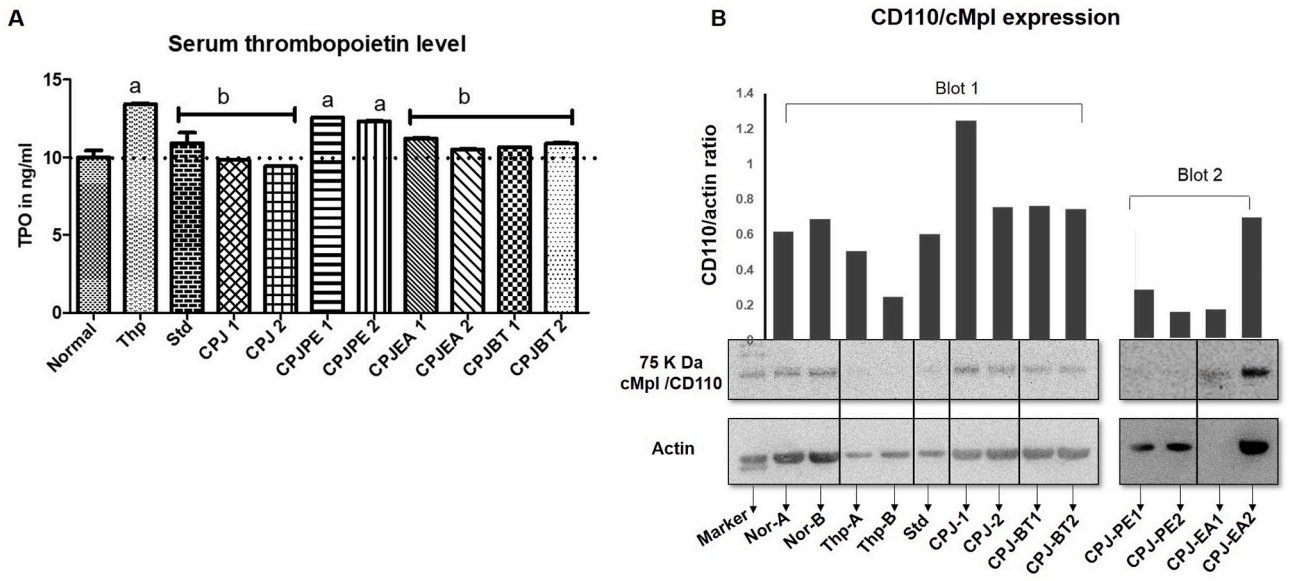

Fig. 6. ***C. papaya*** **leaf juice and Fractions CPJ-EA and CPJ-BT normalized the serum Thrombopoietin level.** Serum thrombopoietin, an indicator of platelet destruction is elevated in cyclophosphamide administered rats compared to control ones. The elevated serum thrombopoietin was significantly decreased in rats treated with CPJ or CPJ-EA and CPJ-BT. However, CPJ-PE failed to produce similar effect(A). CD110/cMpl is a receptor for thrombopoietin. Exposure of rats to cyclophosphamide reduced the CD110/cMpl (75 kDa) expression by promoting the destruction of platelets. However treatment with CPJ, and the Fractions CPJ-BT significantly improved (upregulated the expression of) the CD110 level. β-actin was used as control for protein loading (B). All the values are expressed as Mean ± SEM, (n = 2). Data was analyzed by One way ANOVA with Tukey's post hoc test for multiple comparison. $^{a}P<0.05$ compared to Normal and $^{b}P < 0.05$ compared to disease control (Thp).

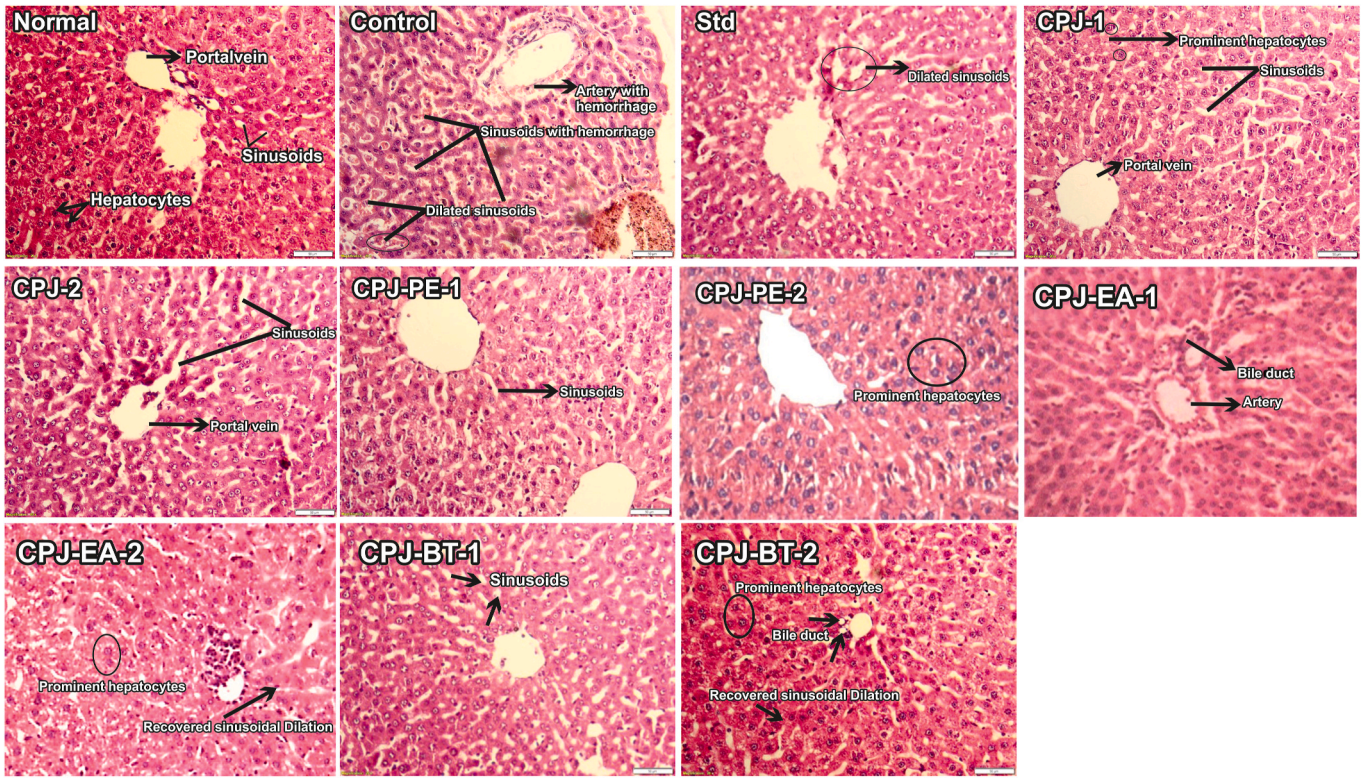

Fig. 7. Histopathological observation of rat liver tissue.
Histological examination of H and E stained section of liver (20X magnification, scale 50 μm) showed sinusoidal damage and hemorrhage in the rats receiving cyclophosphamide. Un injected control group had normal histology and sinusoids (A). However, diseased control thrombocytopenic group exhibited dilated sinusoids and hemorrhage in artery as well as sinusoids (B). Rats treated with standard drug (hydrocortisone) had dilated sinusoids, while the ones administered with CPJ 100 mg/kg and 200 mg/kg body weight showed decreased sinusoidal damage (D & E). Rats exposed to CPJ-PE exhibited recovery in sinusoidal damage and high density of hepatocytes (F & G). Ethyl acetate fraction treated rats showed less sinusoidal space and normal density of hepatocytes (H & I). The rats treated with butanol fraction exhibited well-defined artery and bile duct, slight increased sinusoidal space near artery, recovery of the sinusoidal damage and prominent hepatocytes.

is produced in the sinusoidal cells of liver and kidney (Jelkmann, 2001). In general, low platelet count stimulates the production of TPO (Bacon et al., 1995), (Emmons et al., 1996). Hence, serum TPO has been considered as a marker for platelet count (Makar et al., 2013). However, the correlation between TPO and thrombocytopenia is still unclear. TPO concentration in rats was 10.02 ± 0.42 ng/ml. TPO level was increased to 13.42 ± 0.05 in rats receiving cyclophosphamide. A significant decrease in TPO level was observed in rats treated with standard drug hydrocortisone, and test samples CPJ and CPJ Fractions (Fig. 6A).

3.10. CPJ and CPJ-BT elevated the expression of CD110/cMpl

CD110 (Cluster Differentiation 110), also known as myeloproliferative leukemia protein (cMpl), is a key transmembrane protein responsible for platelet formation (Machlus and Italiano, 2013). The ligand for CD110 is thrombopoietin. Studies have demonstrated decreased expression of CD110 in thrombocytopenic cells. Therefore, in this study, the expression of CD110 was measured in the platelets collected from rats that were exposed to cyclophosphamide and the thrombocytopenic rats treated with CPJ and CPJ Fractions, using Western blotting. All thrombocytopenic rats had decreased expression of CD110 (Fig. 6B, Lanes Thp A and Thp B). Rats treated with CPJ and CPJ-BT showed very high CD110 expression, which is much higher even than normal group (Fig. 6B, Lanes CPJ1, CPJ2, CPJ-BT1, CPJ-BT2). However, CPJ-PE (Lanes CPJ-PE1 and CPJ-PE2) could not increase the CD110 expression. Due to the fixed number of wells in a gel, two gels were used to analyze the samples. The blots were analyzed for the expression and results shown in Fig. 6B.

3.11. CPJ and CPJ fractionsrestored cyclophosphamide-induced thrombocytopenia-associated histological changes in liver and kidney

Cyclophosphamide is a well-known inducer of thrombocytopenia (Aster and Bougie, 2007). Several studies have demonstrated that treatment with cyclophosphamide might cause hepato- and nephrotoxicity in rats (Zarei and Shivanandappa, 2013), (Rehman et al., 2012). In order to test whether CPJ and CPJ Fractions could protect liver and kidney from cyclophosphamide-induced toxicity, organs from the rats were collected (from the efficacy experiment) and morphology and architecture examined after staining with hematoxylin and eosin (Fig. 7). Histological examination using microscope showed sinusoidal damage and hemorrhage in the cyclophosphamide exposed rats. Treatment of rats with 100 mg/kg body weight CPJ had minimal recovery, however, 200 mg/kg CPJ could restore the normal morphology in the liver sinusoids. Among the other Fractions, the CPJ-BT treated rats showed better morphological features similar to normal animals (Fig. 7).

Similarly, the nephrotoxicity induced by cyclophosphamide was restored by the administration of CPJ and CPJ fractions (Fig. 8). A well-developed glomerulus was observed in all the groups compared to thrombocytopenic ones. whereas CPJ-BT exhibited a slight hemorrhagic tissue (Fig. 8).

3.12. Treatment of thrombocytopenic rats with Carica papaya leaf juice (CPJ) reduced bone marrow megakaryocytes

Bone marrow is the producer of platelet progenetor cells viz., megakaryocytes (Patel et al., 2005). Megakaryopoiesis is the process that involves the gradual differentiation of immature megakaryoblast progenitors into diploid megakaryocytes, which undergo subsequent process of cytoplasmic maturation leading to platelet release (Sim et al.,

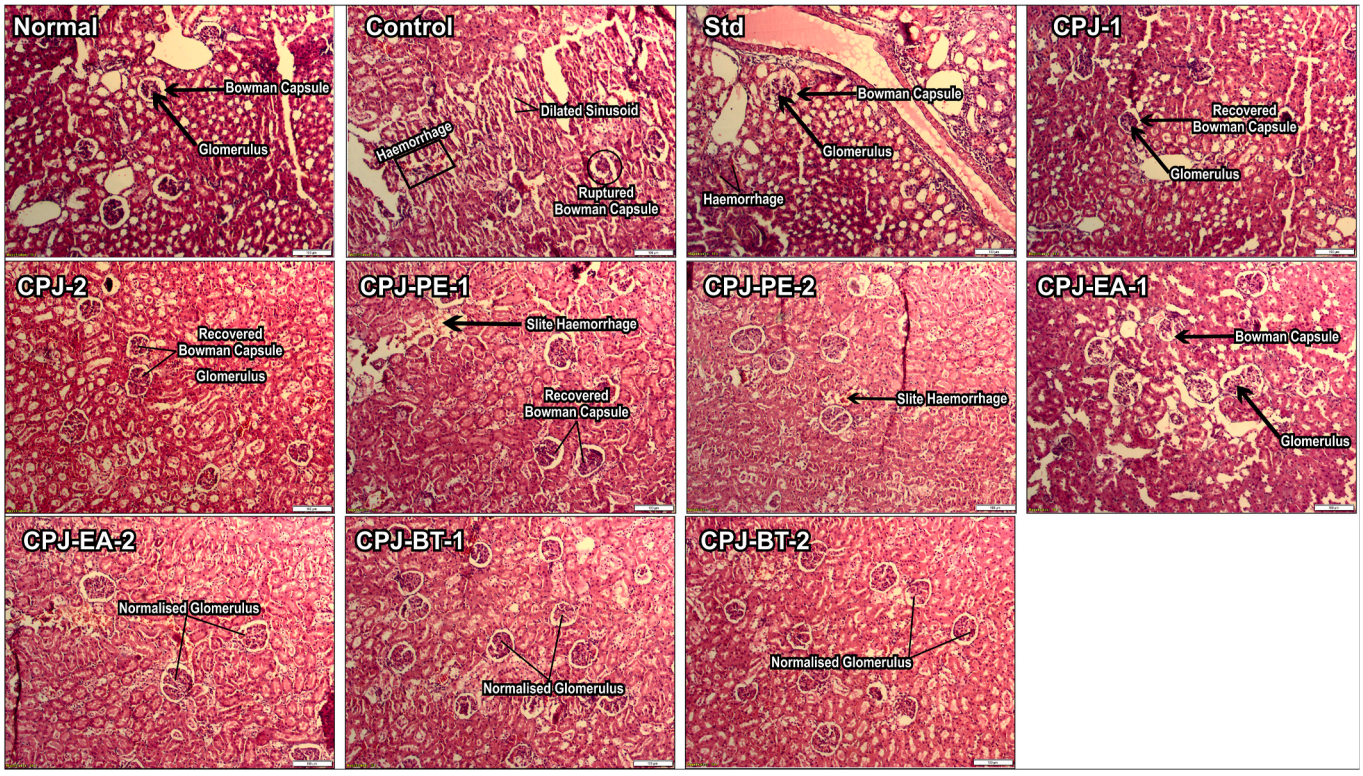

Fig. 8. CPJ and CPJ Fractions restored the normal architecture of rat kidneys. Analysis of H & E stained section (at 40X, Scale 100 μm) of rat kidneys collected from the ones treated with CPJ and CPJ Fractions (A) and the cyclophosphamide exposed thrombocytopenic ones (B). Whereas kidneys of rats treated with CPJ and CPJ Fractions showed normal morphology (A) the Thrombocytopenic ones exhibited glomerular damage and hemorrhage (B). Standard anti-thrombocytopenic drug Hydrocortisone (C) and CPJ-PE, CPJ-EA and CPJ-BT (D, E and F) E. Ethyl acetate showed well developed glomerulus. However, sinusoidal damage and hemorrhage was observed in hydrocortisone treated and butanol administered rats respectively.

2016). Caspase activation in megakaryocytes is the part of apoptotic program, which is activated by cytotoxic agents (Zeuner et al., 2007b). Gunartanum et al., 1984, reported that chemotherapeutic drugs such as cyclophosphamide primarily target the mature megakaryocytes compared to megakaryoblast (de Botton et al., 2002). Low availability of platelets in bloodstream, as observed in diseases such as thrombocytopenia stimulates the production of platelets in bone marrow (Tanum, 1984). Decreased production of platelets is also due to the decreased maturation of megakaryoblast in to megakaryocyte, which is the precursor of platelets. A significant increase in the megakaryoblasts number was observed in rats administered with cyclophosphamide (Fig. 9).

Microscopic examination of bone marrow smear showed normal lobulated megakaryocytes, erythroblast and granulocyte cells. However, more number of megakaryoblast cells were observed in cyclophosphamide administered rats (Fig. 9). Thrombocytopenic animals treated with positive control, hydrocortisone exhibited megakaryocytes and fat cells. Similarly, rats treated with 100 mg/kg CPJ showed fat cells and promegakaryocytes, less cellularity, and high erythroblast numbers. However, rats treated with 200 mg/kg CPJ exhibited mature megakaryocytes with hypercellularity, Bard neutrophil cells (arrow) and increased erythroblast cells (pink colored with arrow head). Likewise, rats exposed to CPJ-PE displayed lobulation in megakaryocytic cells, hypercellularity with Bard neutrophil cells. 200 mg/kg CPJ-PE2 increased the number of megakaryocytes with cytoplasmic vacuoles. Ethyl acetate fraction CPJ-EA showed hypercellularity with megakaryocytes at 100 mg/kg and clear lobulated mature megakaryocytes at 200 mg/kg. The butanol fraction CPJ-BT showed less hypercellularity with mature megakaryocytes at lower dose compared to fully matured megakaryocytes at higher dose.

4. Discussion

Drug-induced thrombocytopenia in chemotherapy is mainly caused by bone marrow toxicity or by liver damage (Zeuner et al., 2007a). Chemotherapeutic drugs while destroying rapidly proliferating cancer cells also affect normal ones such as cells from bone marrow, which are involved in blood formation, cells in the hair follicles or cells in the mouth and intestines (Liebman, 2014). Due to bone marrow toxicity, the production of platelets is retarded, which leads to the induction of thrombocytopenia (Wang et al., 2006).(Zhai et al., 2018). Thrombopoietin is one of the most important cytokines implicated in the formation of platelets from megakaryopoiesis (Matsumura and Kanakura, 2002). TPO is usually produced in sinusoidal cells of the liver and kidney (Cardier and Dempsey, 1998). Sinusoidal dilation by chemotherapy reduces TPO production as observed in the thrombocytopenia patients (Goulis et al., 1999). To date, no effective treatment exists for treating thrombocytopenia. The existing therapeutic strategies such as treatment with interleukin 11 and recombinant thrombopoietin injection are expensive and known to cause side effects like hepatic dysfunction and reticulin fibrosis of bone marrow.

Dengue fever is one of the major health concerns currently in India as well as in other developing countries. During Dengue fever, the number of platelets decreases significantly and cause death. Data from the National Vector Borne Disease Control Programmed (NVBDCP) and

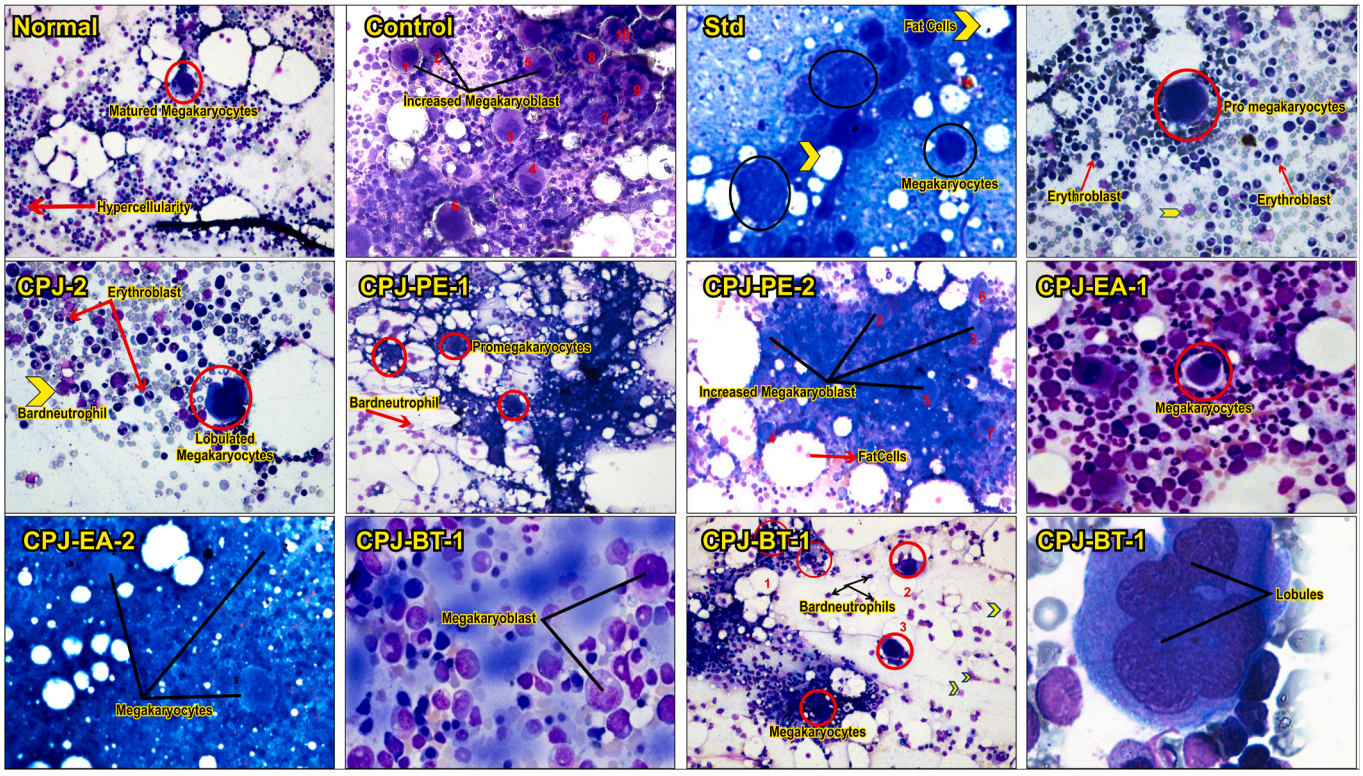

Fig. 9. Deformed morphological changes observed in the bone marrow megakaryocytes were reduced by the administration of CPJ and CPJ extracts. Microscopic examination of bone marrow smear of Rats at 100x (oil immersion) showed a normal lobulated megakaryocytes, hypercellularity and erythroblast and granulocytes cells (A). Thrombocytopenic rats showed more number of megakaryoblast cells. Treatment of rats with standard hydrocortisone showed megakaryocytes (circled ones) and fat cells (pointed with arrow head). D) 100 mg/kg CPJ treated group exhibited high fats cells and promegakaryocytes (circle) and less cellularity, high erythroblast cells (arrow). The rats administered with 200 mg/kg CPJ showed mature megakaryocytes with hypercellularity, Bard neutrophil cells (arrow) and increased erythroblasts (pink colored with arrow head) (E). CPJ-PE1 treated group exhibited lobulation in megakaryocytic cells (circle), hypercellularity with, Bard neutrophil cells (long arrow) (F). CPJ-PE (200 mg/kg) treated group showed increased number of megakaryocytes with cytoplasmic vacuoles (G). 100 mg/kg CPJ-EA showed hypercellularity with megakaryocytes, whereas 200 mg/kg ones exhibited clear lobulated mature megakaryocytes (H). Similarly, CPJ-BT (100 mg/kg and 200 mg/kg body weight) showed less hypercellularity with mature megakaryocytes (I & J). Zoomed version of matured lobulated megakaryocytes in CPJ (200 mg/kg body weight) treated group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

National Health Profile **2018**, stated that the number of deaths due to dengue fever has increased from <60,000 cases in 2009 to 188,401 in 2017 (Abinaya et al., 2018). Therefore, necessary preventive and treatment measures need to be taken to control this disease. A better understanding of this disease at molecular level helps to develop more effective treatment strategies. Hence, in this study, the efficacy of *carica papaya* leaf Fractions in improving the platelet count has been evaluated by examining the levels of serum TPO and its receptor cMpl1 expression in the platelets.

Since *Carica papaya* leaves are known to prevent dengue fever mediated damage to platelets, identifying a chemical compound from this plant source is highly significant (Manohar, 2013) Although significant contributions were made in this direction, majority of the studies have done on the efficacy of Carica papaya leaf juice as a whole, but identification of the phytochemical responsible for that is limited (Sathyapalan et al., 2020; Sharma et al., 2019). In addition, mechanism of action of either the extract or the isolated compounds are also not fully elucidated. Hence, bio guided fractions were generated and partially purified using graded (from hydrophobic to hydrophilic) solvent system mediated isolation procedure (Jagtap et al., 2019). Further all the fractions were subjected to evaluate the mechanism of action of papaya leaf extract and the isolated fraction was studied.

Cyclophosphamide successfully induced thrombocytopenia in rats. Spontaneous bleeding was observed from the nose, beginning from the 7th day due to decreased platelet count (Anjum et al., 2017) (Abe et al., 2016). *Carica papaya* leaf juice and the Fractions isolated exhibited platelet enhancement in the order of CPJ > CPJ-BT > CPJ-EA. Since phenolic acids have been shown to protect the platelets from degradation, an attempt was made to identify key phenolic acids present in the extracts. As predicted, the Fractions were found to contain phenolics and alkaloids, which might be responsible for platelet protection (Anjum et al., 2017). A study by Zunar et al., (2016), reported that alkaloids of petroleum ether and ethyl acetate fraction of *Carica papaya* were responsible for platelet enhancement in busulfan-induced thrombocytopenia. In a separate study Zunar and Anjum et al., 2017, reported the isolation of carpain from papaya leaf juice and stated that carpain might be responsible for platelet protection (Anjum et al., 2017; Zunjar et al., 2016). Data from our study identified potent antioxidant ability of CPJ and its Fractions. Phenolic acid rich Fractions and Fractions exhibited better protection of platelets (Santhakumar et al., 2014). Supporting our data, phenolics have been shown to interact with the receptor-like GPIIb and GPIIIa, which are present on the surface of the platelets responsible for the platelet activating pathway (Ludovici et al., 2018; Natella et al.,).

Interestingly, CPJ and the Fractions have restored the expression of CD110, which was reduced by the administration of cyclophosphamide. Studies have shown decreased CD110 expression in thrombocytopenic group compared to normal healthy controls. Decreased CD110 was also observed in many cancer patients undergoing treatment (Wahid and Atmakusuma, 2017).

5. Conclusion

In conclusion, LC-MS and RP-HPLC data from our study report that solvent Fractions of *Carica papaya* leaf contain phenolic acids, flavonoids and carpaine which may responsible for the platelet count enhancement in animals. Mechanistically, the *Carica papaya* leaf Fractions CPJ and CPJ-BT have increased the expression of cMpl receptor in animals thereby escalated the platelet count. Further studies are warranted to determine the detailed molecular mechanisms of platelet enhancement by CPJ and CPJ-BT. In addition, studies are also warranted to check whether the platelet enhancement effect by CPJ and CPJ-BT is due to a mixture of various compounds present in the extract or any particular compound(s) in the extract/fraction can also produce similar result.

Authors contributions

NC, VB, MA: Conceived, Planned and conduct the experiment, MK: Contributed in Extraction and fractionation of the selected Plant. SM: Contributed in experimental design, data analysis and manuscript preparation SV: Contributed in experimental design, data analysis and manuscript writing JK: Contributed in Histopathological analysis of animal tissues.

Acknowledgements

Authors would like to thank Dr. Divya P. Kumar, (DBT-Ramalingaswamy Fellow), Assistant Professor, Department of Biochemistry, JSS Medical College, JSS Academy of Higher Education & Research, Mysore, Karnataka, India for assisting in language corrections. Authors would like to thank JSS College of Pharmacy and JSS Academy of Higher education and research to provide the platform to conduct the research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.114074.

References

Abe, T., Kono, S., Ohnuki, T., Hishikawa, S., Kunita, S., Hanazono, Y., 2016. A swine model of acute thrombocytopenia with prolonged bleeding time produced by busulfan. Exp. Anim. 65 (4), 345–351. https://doi.org/10.1538/expanim.16-0022 ·.

Abinaya, R., Velavan, A., Purty, A.J., Murugan, N., 2018. Assessment of environmental factors associated with dengue spread in an urban area of Puducherry. Int J Community Med Public Health 5 (7), 3020–3023. https://doi.org/10.18203/2394-6040.ijcmph20182641.

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/S0076- 6879(84)05016-3.

Ahlmann, M., Hempel, G., 2016. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Canc. Chemother. Pharmacol. 78 (4), 661–671. https://doi.org/10.1007/s00280-016-3152-1.

Akhter, T., Khan, M.I., Eva, E.O., 2014. Comparative evaluation of platelet augmentation activity of Carica papaya leaf juice and hydrocortisone in thrombocytopenic rats. Bangladesh J. Physiol. Pharmacol. 30 (2), 32–40. https://doi.org/10.3329/bjpp.v30i2.2268.

Anjum, V., Arora, P., Ansari, S.H., Najmi, A.K., Ahmad, S., 2017. Antithrombocytopenic and immunomodulatory potential of metabolically characterized aqueous extract of Carica papaya leaves. Pharm. Biol. 55 (1), 2043–2056. https://doi.org/10.1080/13880209.2017.1346690 ·.

Aravind, S., Wu, Y., Ranganathan, G., Nalysnyk, L., 2008. Burden of thrombocytopenia in cancer patients undergoing chemotherapy: a retrospective database analysis. Am Soc Hematology 11 (112), 4545–4556. https://doi.org/10.1182/blood.V112.11.4545.4545.

Arya, S.C., Agarwal, N., 2014. Apropos: effects of papaya leaves on thrombocyte counts in dengue–a case report. J. Pakistan Med. Assoc. 64 (8), 979–981.

Aster, R.H., Bougie, D.W., 2007. Drug-induced immune thrombocytopenia. N. Engl. J. Med. 357 (6), 580–587. https://doi.org/10.1111/j.1538-7836.2009.03360.x.

Azeredo, E.L.d., Monteiro, R.Q., de-Oliveira Pinto, L.M., 2015. Thrombocytopenia in dengue: interrelationship between virus and the imbalance between coagulation and fibrinolysis and inflammatory mediators. Mediat. Inflamm. 2015 (1), 1–16. https://doi.org/10.1155/2015/313842.

Bacon, C.M., Justin Tortolani, P., Shimosaka, A., Rees, R.C., Longo, D.L., O'Shea, J.J., 1995. Thrombopoietin (TPO) induces tyrosine phosphorylation and activation of STAT5 and STAT3. FEBS Lett. 370 (1–2), 63–68. https://doi.org/10.1016/0014-5793(95)00796-C.

Balahoroğlu, R., Dülger, H., Özbek, H., Bayram, İ., Şekeroğlu, M., 2008. Protective effects of antioxidants on the experimental liver and kidney toxicity in mice. Eur. J. Gen. Med. 5 (3), 157–164. https://doi.org/10.29333/ejgm/82598.

Bashawri, L.A., Ahmed, M.A., 2007. The approach to a patient with a bleeding disorder: for the primary care physician. J Family Community Med 14 (2), 53–58.

Bhatia, M., Davenport, V., Cairo, M.S., 2007. The role of interleukin-11 to prevent chemotherapy-induced thrombocytopenia in patients with solid tumors, lymphoma, acute myeloid leukemia and bone marrow failure syndromes. Leuk. Lymphoma 48 (1), 9–15. https://doi.org/10.1080/10428190600909115.

Boligon, A., Athayde, M., 2014. Importance of HPLC in analysis of plants extracts. Austin Chromatogr. 1 (3), 2–4.

Bordoloi, P., Devi, D., Dasgupta, S., 2016. A comparative study of the platelet augmentation potential of leaf extracts of Psidium guajava with Carica papaya in thrombocytopenic rats. Scholars J. Appl. Med. Sci. 4 (1), 2774–2782. https://doi.org/10.21276/sjams.2016.4.8.10.

Bystrom, Laura M., 2008. Characterization of phenolics by LC-UV/vis, LC-MS/MS and sugars by GC in Melicoccus bijugatus Jacq. 'Montgomery' fruits. Food Chem. 111 (4), 1017–1024. https://doi.org/10.1016/j.foodchem.2008.04.058.
Cardier, J.E., Dempsey, J., 1998. Thrombopoietin and its receptor, c-mpl, are constitutively expressed by mouse liver endothelial cells: evidence of thrombopoietin as a growth factor for liver endothelial cells. Blood 91 (3), 923–929.
Carson, J.L., Stanworth, S.J., 2017. Anemia and bleeding in thrombocytopenic patients. Blood 130 (10), 1178–1179. https://doi.org/10.1182/blood-2017-07-795922.
Chow, L., Aslam, R., Speck, E.R., Kim, M., Cridland, N., Webster, M.L., Chen, P., Sahib, K., Ni, H., Lazarus, A.H., 2010. A murine model of severe immune thrombocytopenia is induced by antibody-and CD8+ T cell–mediated responses that are differentially sensitive to therapy. Blood 115 (6), 1247–1253. https://doi.org/10.1182/blood-2009-09-244772.
Cox, P.J., Abel, G., 1979. Cyclophosphamide cystitis: studies aimed at its minimization. Biochem. Pharmacol. 28 (24), 3499–3502. https://doi.org/10.1016/0006-2952(79)90390-3.
Dameshek, W., Miller, E.B., 1946. The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism. Blood 1 (1), 27–51. https://doi.org/10.1182/blood.V1.1.27.27.
David, J., Kuter, M.D., 2015. Managing thrombocytopenia associated with cancer chemotherapy. Oncol. 29 (4), 1–6.
de Botton, S., Sabri, S., Daugas, E., Zermati, Y., Guidotti, J.E., Hermine, O., Kroemer, G., Vainchenker, W., Debili, N., 2002. Platelet formation is the consequence of caspase activation within megakaryocytes. Blood 100 (4), 1310–1317. https://doi.org/10.1182/blood-2002-03-0686.
Dhurat, R., Sukesh, M.S., 2014. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J. Cutan. Aesthetic Surg. 7 (4), 189–197. https://doi.org/10.4103/0974-2077.150734.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82 (1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6.
Eltantawy, F.M., Sobh, M.A.A., EL-Waseef, A.M., Ibrahim, R.-A.A., Saad, M.A., 2018. Protective effect of Spirulina against cyclophosphamide-induced urotoxicity in mice. Egyptian Journal of Basic and Applied Sciences 5 (3), 191–196. https://doi.org/10.1016/j.ejbas.2018.06.001.
Emmons, R., Reid, D.M., Cohen, R., Meng, G., Young, N., Dunbar, C., Shulman, N., 1996. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 87 (10), 4068–4071.
Etulain, J., Mena, H.A., Meiss, R.P., Frechtel, G., Gutt, S., Negrotto, S., Schattner, M., 2018. An optimised protocol for platelet-rich plasma preparation to improve its angiogenic and regenerative properties. Sci. Rep. 8 (1), 1513–1528.
Gale, A.J., 2011. Continuing education course# 2: current understanding of hemostasis. Toxicol. Pathol. 39 (1), 273–280. https://doi.org/10.1177/0192623310389474.
Garcia-Manzano, A., Gonzalez-Llaven, J., Lemini, C., Rubio-Poo, C., 2001. Standardization of rat blood clotting tests with reagents used for humans. In: Proceedings of the Western Pharmacology Society. The Society, Seattle, Wash, pp. 153–156.
Goulis, J., Chau, T., Jordan, S., Mehta, A., Watkinson, A., Rolles, K., Burroughs, A., 1999. Thrombopoietin concentrations are low in patients with cirrhosis and thrombocytopenia and are restored after orthotopic liver transplantation. Gut 44 (5), 754–758.
Gunde, M.C., Amnerkar, N.D., 2016. Nutritional, medicinal and pharmacological properties of papaya (Carica papaya Linn.): a review. J Innov Pharm Biol Sci 3 (1), 162–169.
Gupta, N.K., Bansal, S.B., Jain, U.C., Sahare, K., 2013. Study of thrombocytopenia in patients of malaria. Tropenmed. Parasitol. 3 (1), 58–61. https://doi.org/10.4103/2229-5070.113914.
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. J. Med. Plants Res. 5 (10), 1867–1872.
Hamad, O.A., Nilsson, P.H., Wouters, D., Lambris, J.D., Ekdahl, K.N., Nilsson, B., 2010. Complement component C3 binds to activated normal platelets without preceding proteolytic activation and promotes binding to complement receptor 1. J. Immunol. 184 (5), 2686–2692. https://doi.org/10.4049/jimmunol.0902810.
Hassan, M.N., Waller, E.K., 2015. Treating chemotherapy-induced thrombocytopenia: is it time for oncologists to use thrombopoietin agonists? Oncology (Williston Park) 29 (4), 295–296.
Hoffman, M., 2003. Remodeling the blood coagulation cascade. J. Thromb. Thrombolysis 16 (1–2), 17–20. https://doi.org/10.1023/B:THRO.0000014588.95061.28.
Ismail, Z., Halim, S.Z., Abdullah, N.R., Afzan, A., Abdul Rashid, B.A., Jantan, I., 2014. Safety evaluation of oral toxicity of carica papaya linn. Leaves: a subchronic toxicity study in Sprague dawley rats. Evid Based Complement Alternat Med 2014 (1), 1–10. https://doi.org/10.1155/2014/741470.
Jayasinghe, C.D., Gunasekera, D.S., De Silva, N., Jayawardena, K.K.M., Udagama, P.V., 2017. Mature leaf concentrate of Sri Lankan wild type Carica papaya Linn. modulates nonfunctional and functional immune responses of rats. BMC Compl. Alternative Med. 17 (1), 230. https://doi.org/10.1186/s12906-017-1742-z.
Jagtap, N.S., Wagh, R.V., Chatli, M.K., Kumar, P., Malav, O., Mehta, N., 2019. Optimisation of extraction protocol for Carica papaya L. to obtain phenolic rich phyto-extract with prospective application in chevon emulsion system. J. Food Sci. Technol. 56 (1), 71–82. https://doi.org/10.1007/s13197-018-3456-8.
Jelkmann, W., 2001. The role of the liver in the production of thrombopoietin compared with erythropoietin. Eur. J. Gastroenterol. Hepatol. 13 (7), 791–801. https://doi.org/10.1097/00042737-200107000-00006.
Jose, A., Chaitanya, M.V.N.L., Kannan, E., Madhunapantula, S.V., 2018. Tricaproin isolated from simarouba glauca inhibits the growth of human colorectal carcinoma cell lines by targeting class-1 histone deacetylases. Front. Pharmacol. 9 (127), 1–19. https://doi.org/10.3389/fphar.2018.00127.
Kamal, A.H., Tefferi, A., Pruthi, R.K., 2007. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clin. Proc. 82 (7), 864–873. https://doi.org/10.4065/82.7.864.
Krishna, K.L., Paridhavi, M., Patel, J.A., 2008. Review on nutritional, medicinal and pharmacological properties of Papaya (Carica papaya Linn.). Indian journal of natural products and research 7 (4), 364–373.
Kristiana, H., Nailufar, F., Winoto, I.L., Tjandrawinata, R.R., 2013. A model of rat thrombocytopenia induced by cyclophosphamide. JFIOnline 6 (3), 12–23. https://doi.org/10.35617/jfi.v6i3.139.
Kumar, B.R., 2017. Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (GLVs). J Pharm Anal 7 (6), 349–364. https://doi.org/10.1016/j.jpha.2017.06.005.
Kumar, K., Murthy, A., Upadhyay, O., 1998. Plants used as antidotes by the tribals of Bihar. Ancient Sci. Life 17 (4), 268–272.
Koley, T.K., Khan, Z., Oulkar, D., Singh, B., Maurya, A., Singh, B., Banerjee, K., 2020. High resolution LC-MS characterization of phenolic compounds and the evaluation of antioxidant properties of a tropical purple radish genotype. Arabian J Chem 13 (1), 1355–1366. https://doi.org/10.1016/j.arabjc.2017.11.007.
Laffi, G., Tarquini, R., Marra, F., 2007. Thrombocytopenia in chronic liver disease: lessons from transplanted patients. J Heptol 47 (5), 625–629. https://doi.org/10.1016/j.jhep.2007.08.006.
Liebman, H.A., 2014. Thrombocytopenia in cancer patients. Thromb. Res. 133 (1), 63–69. https://doi.org/10.1016/S0049-3848(14)50011-4.
Ludovici, V., Barthelmes, J., Nagele, M.P., Flammer, A.J., Sudano, I., 2018. Polyphenols: anti-platelet nutraceutical? Curr. Pharmaceut. Des. 24 (2), 146–157. https://doi.org/10.2174/1381612823666171109104600.
Machlus, K.R., Italiano, J.E., 2013. The incredible journey: from megakaryocyte development to platelet formation. J. Cell Biol. 201 (6), 785–796. https://doi.org/10.1083/jcb.201304054.
Makar, R.S., Zhukov, O.S., Sahud, M.A., Kuter, D.J., 2013. Thrombopoietin levels in patients with disorders of platelet production: diagnostic potential and utility in predicting response to TPO receptor agonists. Am. J. Hematol. 88 (12), 1041–1044. https://doi.org/10.1002/ajh.23562.
Mann, K.G., Orfeo, T., Butenas, S., Undas, A., Brummel-Ziedins, K., 2009. Blood coagulation dynamics in haemostasis. Hämostaseologie 29 (1), 7–16.
Manohar, P.R., 2013. Papaya, dengue fever and Ayurveda. Ancient Sci. Life 32 (3), 131–133. https://doi.org/10.4103/0257-7941.122994.
Matsumura, I., Kanakura, Y., 2002. Molecular control of megakaryopoiesis and thrombopoiesis. Int. J. Hematol. 75 (5), 473–483. https://doi.org/10.1007/BF02982109.
McElroy, A., 2015. Understanding bleeding in ebola virus disease. Clin. Adv. Hematol. Oncol. 13 (1), 29–31.
McElroy, P.L., Wei, P., Buck, K., Sinclair, A.M., Eschenberg, M., Sasu, B., Molineux, G., 2015. Romiplostim promotes platelet recovery in a mouse model of multicycle chemotherapy-induced thrombocytopenia. Exp hematol 43 (6), 479–487. https://doi.org/10.1016/j.exphem.2015.02.004.
MedCrave Venkataraman, N., Pamukuntla, S., Banoth, J., 2015. Platelet augmentation activity of Andrographis paniculata extract and andrographolide against cyclophosphamide induced thrombocytopenia in rats. Pharm Pharmacol Int J 2 (4), 126–131.
Merli, P., Strocchio, L., Vinti, L., Palumbo, G., Locatelli, F., 2015. Eltrombopag for treatment of thrombocytopenia-associated disorders. Expet Opin. Pharmacother. 16 (14), 2243–2256. https://doi.org/10.1517/14656566.2015.1085512.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10), 3170–3175.
Mitchell, O., Feldman, D.M., Diakow, M., Sigal, S.H., 2016. The pathophysiology of thrombocytopenia in chronic liver disease. Hepat Med 8 (15), 39–50. https://doi.org/10.2147/HMER.S74612.
Moore, F., Urda, G., Krishna, G., Theiss, J., 1995. An in vivo/in vitro method for assessing micronucleus and chromosome aberration induction in rat bone marrow and spleen 1. Studies with cyclophosphamide. Mutat. Res. 335 (2), 191–199. https://doi.org/10.1016/0165-1161(95)90055-1.
Mutlag, S.H., Abdulhussein, A.J., Taher, M.A., Hawi, A.J., 2018. The effect of ethanol on platelet count, bleeding time, and white blood cells in rats. Drug Invent. Today 10 (1), 2600–2604.
Nandini, C., Kumar, S., Jayashree, K., Mruthunjaya, K., Johnson, K., Manjula, S., 2016. Platelet enhancing effect of eclipta alba in alcohol induced thrombocytopenic thrombocytopenic rats. Int J Pharm Sci Res 7 (5), 2219–2230. https://doi.org/10.13040/IJPSR.0975-8232.7(5).2219-30.
Natella, F., Nardini, M., Virgili, F., Scaccini, C. Role of dietary polyphenols in the platelet aggregation network - a review of the in vitro studies. Plateltes 18(3), 224-243. https://doi.org/10.1080/09537100601078083.
NaveenKumar, S.K., Thushara, R.M., Sundaram, M.S., Hemshekhar, M., Paul, M., Thirunavukkarasu, C., Basappa, Nagaraju, G., Raghavan, S.C., Girish, K.S., Kemparaju, K., Rangappa, K.S., 2015. Unconjugated bilirubin exerts pro-apoptotic effect on platelets via p38-MAPK activation. Sci. Rep. 5, 1–15.
Neschadim, A., Branch, D.R., 2016. Mouse models for immune-mediated platelet destruction or immune thrombocytopenia (ITP). Curr. Protoc. Im. 113 (1), 15–30. https://doi.org/10.1002/0471142735.im1530s113.
Newland, A., 2009. Thrombopoietin receptor agonists in the treatment of thrombocytopenia. Curr. Opin. Hematol. 16 (5), 357–364. https://doi.org/10.1097/MOH.0b013e32832e06e4.

Nie, H., Li, K.-y., Zhang, X.-q., Feng, X.-y., Yang, D.-r., Wu, Y.-s., Zhou, J.-y., Ye, W.-c., 2009. Establishment of a mouse thrombocytopenia model induced by cyclophosphamide. Zool. Res. 30 (6), 645–652.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
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. https://doi.org/10.1016/j.jep.2009.11.024.
Park, Y.A., Waldrum, M.R., Marques, M.B., 2010. Platelet count and prothrombin time help distinguish thrombotic thrombocytopenic purpura–hemolytic uremic syndrome from disseminated intravascular coagulation in adults. Am. J. Clin. Pathol. 133 (3), 460–465. https://doi.org/10.1309/AJCPPNF63FLIORCI.
Patel, S.R., Hartwig, J.H., Italiano, J.E., 2005. The biogenesis of platelets from megakaryocyte proplatelets. J. Clin. Invest. 115 (12), 3348–3354. https://doi.org/10.1172/JCI26891.
Patil, S., Shetty, S., Bhide, R., Narayanan, S., 2013. Platelet agumentation activity of carica papaya aquoes extract on rat. J. Pharmacogn. Phytochem. 1 (5), 57–60.
Pratico, D., Iuliano, L., Pulcinelli, F.M., Bonavita, M.S., Gazzaniga, P.P., Violi, F., 1992. Hydrogen peroxide triggers activation of human platelets selectively exposed to nonaggregating concentrations of arachidonic acid and collagen. J. Lab. Clin. Med. 119 (4), 364–370.
Psaila, B., Lyden, D., Roberts, I., 2012. Megakaryocytes, malignancy and bone marrow vascular niches. J. Thromb. Haemostasis 10 (2), 177–188. https://doi.org/10.1111/j.1538-7836.2011.04571.x.
Ray, S., Pandit, B., Das, S., Chakraborty, S., 2011. Cyclophosphamide-induced lipid peroxidation and changes in cholesterol content: protective role of reduced glutathione. Iran. J. Pharm. Res. (IJPR) 7 (4), 255–267.
Rehman, M.U., Tahir, M., Ali, F., Qamar, W., Lateef, A., Khan, R., Quaiyoom, A., Sultana, S., 2012. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of Ellagic acid. Mol. Cell. Biochem. 365 (1–2), 119–127. https://doi.org/10.1007/s11010-012-1250-x.
Reilly, M.P., McKenzie, S.E., 2002. Insights from mouse models of heparin-induced thrombocytopenia and thrombosis. Curr. Opin. Hematol. 9 (5), 395–400. https://doi.org/10.1097/00062752-200209000-00002.
Rice, L., 2009. Treatment of immune thrombocytopenic purpura: focus on eltrombopag. Biologics 3 (2), 151–162. https://doi.org/10.2147/btt.2009.2984.
Santhakumar, A.B., Bulmer, A.C., Singh, I., 2014. A review of the mechanisms and effectiveness of dietary polyphenols in reducing oxidative stress and thrombotic risk. J. Hum. Nutr. Diet. 27 (1), 1–21. https://doi.org/10.1111/jhn.12177.
Sathasivam, K., Ramanathan, S., Mansor, S.M., Haris, M.R.M.H., Wernsdorfer, W.H., 2009. Thrombocyte counts in mice after the administration of papaya leaf suspension. Wien Klin. Wochenschr. 121 (3), 19–22. https://doi.org/10.1007/s00508-009-1229-0.
Samson, M., Fraser, W., Lebowitz, D., 2019. Treatments for primary immune thrombocytopenia: a review. Cureus 11 (10). https://doi.org/10.7759/cureus.5849.
Sharma, N., Mishra, D., 2014. Papaya leaves in dengue fever: is there scientific evidence. Indian Pediatr. 51 (4), 324–325.
Sathyapalan, D.T., Padmanabhan, A., Moni, M., P-Prabhu, B., Prasanna, P., Balachandran, S., Trikkur, S.P., Jose, S., Edathadathil, F., Anilkumar, J.O., 2020. Efficacy & safety of Carica papaya leaf extract (CPLE) in severe thrombocytopenia (≤ 30,000/μl) in adult dengue–Results of a pilot study. PloS One 15 (2), e0228699. https://doi.org/10.1371/journal.pone.0228699.
Siddique, O., Sundus, A., Ibrahim, M.F., 2014. Effects of papaya leaves on thrombocyte counts in dengue–a case report. J. Pakistan Med. Assoc. 64 (3), 364–366.
Sim, X., Poncz, M., Gadue, P., French, D.L., 2016. Understanding platelet generation from megakaryocytes: implications for in vitro–derived platelets. Blood 127 (10), 1227–1233. https://doi.org/10.1182/blood-2015-08-607929.
Snyder, L.S., Heigh, R.I., Anderson, M.L., 1993. Cyclophosphamide-induced hepatotoxicity in a patient with Wegener's granulomatosis. Mayo Clin. Proc. 68 (12), 1203–1204. https://doi.org/10.1016/s0025-6196(12)60074-3.
Subenthiran, S., Choon, T.C., Cheong, K.C., Thayan, R., Teck, M.B., Muniandy, P.K., et al., 2013. Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue hemorrhagic fever. Evid Based Complement Alternat Med 2013 (1), 1–7. https://doi.org/10.1155/2013/616737.
Shokrzadeh, M., Ahmadi, A., Naghshvar, F., Chabra, A., Jafarinejhad, M., 2014. Prophylactic efficacy of melatonin on cyclophosphamide-induced liver toxicity in mice. BioMed Res. Int. 2014 (1), 1–6. https://doi.org/10.1155/2014/470425.
Subba Rao, M.V., Muralikrishna, G., 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, Eleusine coracana Indaf-15). J. Agric. Food Chem. 50 (4), 889–892. https://doi.org/10.1021/jf011210d.
Seal, T., 2016. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, Sonchus arvensis and Oenanthe linearis of North-Eastern region in India. J. Appl. Pharmaceut. Sci. 6 (2), 157–166. https://doi.org/10.7324/JAPS.2016.60225.
Tahir, N., Zaheer, Z., Kausar, S., Chiragh, S., 2014. Prevention of fall in platelet count by carica papaya leaf juice in carboplatin induced thrombocytopaenia in mice. Biomed 30 (1), 21–24.
Tanum, G., 1984. The megakaryocyte DNA content and platelet formation after the sublethal whole body irradiation of rats. Blood 63 (4), 917–920.
ten Berg, M.J., van den Bemt, P.M., Shantakumar, S., Bennett, D., Voest, E.E., Huisman, A., van Solinge, W.W., Egberts, T.C., 2011. Thrombocytopenia in adult cancer patients receiving cytotoxic chemotherapy. Drug Saf. 34 (12), 1151–1155. https://doi.org/10.2165/11594310-000000000-00000.
Terrell, D.R., Neunert, C.E., Cooper, N., Heitink-Pollé, K.M., Kruse, C., Imbach, P., Kühne, T., Ghanima, W., 2020. Immune thrombocytopenia (ITP): current limitations in patient management. Medicina 56 (12), 667.
Uhl, L., Assmann, S.F., Hamza, T.H., Harrison, R.W., Gernsheimer, T., Slichter, S.J., 2017. Laboratory predictors of bleeding and the effect of platelet and RBC transfusions on bleeding outcomes in the PLADO trial. Blood 130 (10), 1247–1258. https://doi.org/10.1182/blood-2017-01-757930.
Vadhan-Raj, S., 2009. Management of chemotherapy-induced thrombocytopenia: current status of thrombopoietic agents. Semin. Hematol. 46 (1), 26–32. https://doi.org/10.1053/j.seminhematol.2008.12.007.
Vianello, F., Vettore, S., Tezza, F., Toni, L.D., Scandellari, R., Sambado, L., Treleani, M., Fabris, F., 2014. Serum thrombopoietin and cMpl expression in thrombocytopenia of different etiologies. Hematol. Rep. 6 (1), 685–696. https://doi.org/10.4081/hr.2014.4996.
Vinayakamurthy, S., Potluri, R., Shivajirao, P., Singh, R., Pujahari, R., Maniketh, I., 2017. A study of megakaryocyte morphology in bone marrow aspiration smears of cases of thrombocytopenia. Med J DY Patil Univ 10 (1), 51–57. https://doi.org/10.4103/0975-2870.197922.
Visentin, G.P., Liu, C.Y., 2007. 9. Drug induced thrombocytopenia. Hematol. Oncol. Clin. N. Am. 21 (4), 685–696. https://doi.org/10.1016/j.hoc.2007.06.005.
Wahid, I., Atmakusuma, T.D., 2017. Correlation of c-MPL expression and platelet count in thrombocytopenia due to chemotherapy patients. Blood 130 (1), 4863-4863.
Wang, Y., Probin, V., Zhou, D., 2006. Cancer therapy-induced residual bone marrow injury-Mechanisms of induction and implication for therapy. Curr. Canc. Ther. Rev. 2 (3), 271–279.
Weycker, D., Hatfield, M., Grossman, A., Hanau, A., Lonshteyn, A., Sharma, A., Chandler, D., 2019. Risk and consequences of chemotherapy-induced thrombocytopenia in US clinical practice. BMC Canc. 19 (1), 151–165. https://doi.org/10.1186/s12885-019-5354-5.
Yunita, F., Hanani, E.S., Kristianto, J., 2012. The effect of Carica papaya L. leaves extract capsules on platelets count and hematocrit level in dengue fever patient. Int. J. Med. Aromatic Plants 2 (4), 573–578.
Zarei, M., Shivanandappa, T., 2013. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of Decalepis hamiltonii in mice. Food Chem. Toxicol. 57 (2), 179–184. https://doi.org/10.1016/j.fct.2013.03.028.
Zeuner, A., Signore, M., Martinetti, D., Bartucci, M., Peschle, C., De Maria, R., 2007a. Chemotherapy-induced thrombocytopenia derives from the selective death of megakaryocyte progenitors and can be rescued by stem cell factor. Cancer Res 67 (10), 4767–4773.
Zeuner, A., Signore, M., Martinetti, D., Bartucci, M., Peschle, C., Maria, R.D., 2007b. Chemotherapy-induced thrombocytopenia derives from the selective death of megakaryocyte progenitors and can Be rescued by stem cell factor. Cancer Res 67 (10), 4767–4773. https://doi.org/10.1158/0008-5472.CAN-06-4303.
Zhai, X., Zhang, Z., Liu, W., Liu, B., Zhang, R., Wang, W., Zheng, W., Xu, F., Wang, J., Chen, Y., 2018. Protective effect of ALDH2 against cyclophosphamide-induced acute hepatotoxicity via attenuating oxidative stress and reactive aldehydes. Biochem. Biophys. Res. Commun. 499 (1), 93–98. https://doi.org/10.1158/0008-5472.CAN-06-4303.
Zhou, B., Kong, X., Linzer, D.I., 2005. Enhanced recovery from thrombocytopenia and neutropenia in mice constitutively expressing a placental hematopoietic cytokine. Endocrinol 146 (1), 64–70. https://doi.org/10.1210/en.2004-1011.
Zunjar, V., Dash, R.P., Jivrajani, M., Trivedi, B., Nivsarkar, M., 2016. Antithrombocytopenic activity of carpaine and alkaloidal extract of Carica papaya Linn. leaves in busulfan induced thrombocytopenic Wistar rats. J. Ethnopharmacol. 181 (1), 20–25. https://doi.org/10.1016/j.jep.2016.01.035.
Zunjar, V., Mammen, D., Trivedi, B.M., 2015. Antioxidant activities and phenolics profiling of different parts of Carica papaya by LCMS-MS. Nat. Prod. Res. 29 (22), 2097–2099. https://doi.org/10.1080/14786419.2014.986658.

Glossary

Thp: Thrombocytopenia
CYP: Cyclophosphamide
C. papaya: Carica papaya
CPJ: Carica papaya leaf juice
CPJ-PE: Petroleum ether fraction of *Carica Papaya* leaf juice.
CPJ-EA: Ethyl acetate fraction of *Carica Papaya* leaf juice.
CPJ-BT: Butanol fraction of *Carica Papaya* leaf juice.
FDA: Food drug administration
PB: Phosphate buffer
ECL: Chemiluminescence substrate
HRP: Horseradish peroxidase
TBST: Tris buffered saline
BSA: 'Bovine serum 'albumin
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
H_2O_2: Hydrogen peroxide
GSH: Glutathione
SOD: Superoxide dismutase
CAT: Catalase
GPx: Glutathione peroxidase
LPO: Lipid peroxidase
MDA: Malondialdehyde

RBC: Red blood cell
WBC: White blood cell
i.p.: Intraperitoneal
b.wt: Body weight
p.o: Post oral
ROS: Reactive oxygen species
rpm: Revolutions per minute
TCA: Trichloroacetic acid
TBA: Thiobarbituric acid
H&E: Hematoxylin & eosin
NO: Nitric oxide
CIT: Chemotherapy induced thrombocytopenia
TPO: Thrombopoietin
cMpl: Myeloproliferative leukemia protein