Antithrombocytopenic potential of metabolically characterized extract and fractions of *Momordica charantia* L. fruits by *in vivo* study

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Article Info

Abstract

Momordica charantia L. is a popular medicinal plant used in the traditional system of medicine for the management or treatment of various diseases and disorders. This study was aimed to explore the antithrombocytopenic potential of extract and different fractions of *M. charantia* fruits in busulfan-induced thrombocytopenic rats. The hydroalcoholic extract (mother extract) was prepared and further fractionated based on polarity such as n-hexane, dichloromethane (DCM), n-butanol, and aqueous, respectively. Interestingly, the DCM fraction and mother extract were identified as potent antithrombocytopenic agents. DCM fractions and mother extract were metabolically characterized using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS). A total of 08 and 05 metabolites were tentatively identified in the DCM fraction and mother extract, respectively. Moreover, standardized DCM fractions and mother extract of *M. charantia* significantly ($p<0.05$) enhanced platelet count as compared to other fraction like hexane, n-butanol, and aqueous. Among all, the DCM fraction improves overall tested parameters of the blood including RBC, hemoglobin, and neutrophil. Therefore, observed results inferred that *M. charantia* can be used for the management of thrombocytopenia.

1. Introduction

Dengue is one of the major evolving viral diseases that threaten human life and economies throughout the globe. In the recent past, occurrence of dengue increases dramatically across the world. Annually, about 390 million people suffering from dengue infections globally that leads to 25,000 deaths. As per the report published by WHO in 2021, the number of dengue cases was reported at 2.2 million in 2010 but in 2019, the number of cases reached 5.2 million (WHO, 2021). Primarily, dengue weakens the body's immunity and the most common associated symptoms were observed such as headache, hypertension, mental disorders, inflammation, liver inflammation, and thrombocytopenia with or without hemorrhage. In case of severe and long-term occurrence of dengue may damage the liver cause excessive bleeding that leads to human death (Ahmad *et al*., 2019).

Platelets are a type of blood cells produced in the bone marrow that play a key role in the maintenance of hemostasis. It is also involved in blood clotting to prevent blood loss in injury. Thrombocytopenia (low count of platelet/thrombocytes) is associated with prolonged bleeding and clotting time due to deficient platelet counts in the blood and it occurs either by genetic disorders or infections like dengue fever, parvovirus, and bacterial infections like tuberculosis

(Talla 2015). Most of the anticancer agents cause thrombocytopenia as an adverse effect during the period of treatment. Based on the above effect, busulfan was used to induce thrombocytopenia in experimental animals for the evaluation of platelet augmentation activity of prepared extract and fraction (Zunjar *et al.,* 2016). To date, no vaccine or medicament is available to us for the management of dengue-induced thrombocytopenia, hence people are relies on traditional medicine to cure dengue. For millennia, *Momordica charantia* is a very important medicinal plant used for the management of various diseases and disorders. This plant belonging to the family Cucurbitaceae and is commonly known as bitter gourd (Habicht *et al.,* 2011). Cited literature revealed that seeds and fruit of *M. charantia* possess therapeutic effects (Grover and Yadav, 2004; Prasad *et al*., 2006). It offers a huge number of pharmacological values such asantidiabetic, antimicrobial, hepatoprotective, anticancer and anti-inflammatory activities, and many more (Raman and Lau 1996; Virdi *et al*., 2003.; Mada *et al*., 2013; Costa *et al*., 2010; Jagessara *et al.*, 2008).

M. charantia is a good source of natural antioxidants, has shown its activity against oxidant damage (Fernandes *et al.,* 2007; Lucas *et al.* 2010; Xie *et al*., 2010). Leaves and stems ethanol extracts have antiviral effects which inhibit HSV-1 and SINV viruses (Beloin *et al*., 2005; Lee-Huang *et al*., 1990). Dar and his team reported that *M. charantia* elevates platelet counts, hemoglobin and also maintains electrolyte balance (Dar *et al.,* 2014). It also possesses anti-inflammatory effects by the mechanism which inhibits infiltration of neutrophils and NF-κB activation, depressed MMP-9 levels, and ameliorates TNF-α IL-8, and IL-1β levels (Huang *et al*., 2015; Jia *et al.,* 2017). Day-by-day resurgence of herbal preparations increases

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globally but, due to the lack of inadequate regulations, these formulations are not being accepted by the modern world. Therefore, the present study explored to ascertain the antithrombocytopenic effect of *M. charantia* in an experimental animal model of rats along with complete quality control analysis of used extract and fraction. Moreover, the resulting outcome can be used to explore the action mechanism of responsible bioactive metabolites for the management of dengue fever/thrombocytopenic conditions.

2. Materials and Methods

2.1 Plant materials and chemicals

Fresh fruits of *M. charantia* were collected from the Khari Bawali, New Delhi, India, and taxonomic identification was done at CSIR-NISCAIR, New Delhi by Dr. Sunita Garg, Chief Scientist. The sample has been deposited at BNPL, SPER, Jamia Hamdard for future reference (Voucher Specimen No. NISCAIR/RHMD/Consult/2015/2889/82-1). Chemicals used in this study were of analytical grade and purchased from SD Fine Chemicals (India). Busulfan was purchased from Sigma-Aldrich (India) and used for induction of thrombocytopenia in rats.

2.2 Preparation of extract and its fractionation

100 gm whole fruits of *M. charantia* were powdered, extracted with 80% v/v ethanol for 16 h at 80°C using soxhlet apparatus. The filtrate was dried on a water bath, further dried extract was suspended in water (10 g/ml) for 15 min at 45°C. The suspension was obtained, partitioned, and fractionated using *n*-hexane, dichloromethane (DCM), *n*-butanol, and water-based on polarity. The obtained fractions were dried using rota vapor and stored in a well-closed container for further use (Figure 1) (Pisoschi *et al*., 2016).
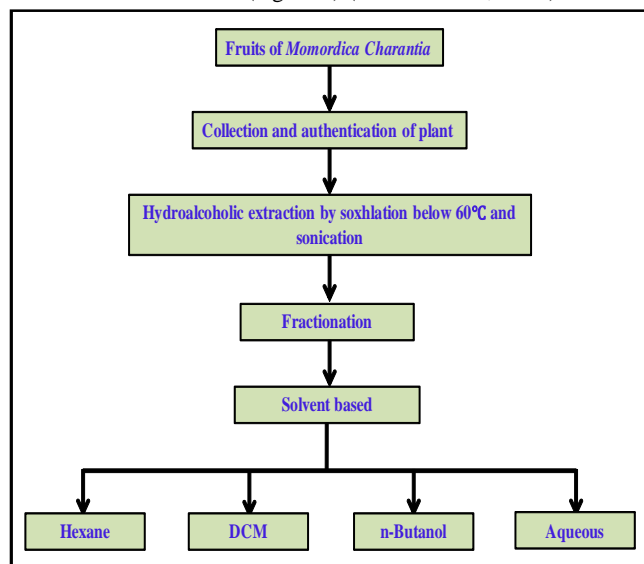
Figure 1: Typical layout for the extraction and their fractionationusing different solvent system.

2.3 UPLC-MS analysis

The samples were prepared in LC-MS grade methanol. Before analysis, the sample was filtered using a syringe filter. Chromatographic separation of metabolites was done using mobile consisting of 0.5% formic acid in water (A) and acetonitrile (B) and BEH C18

(100 mm × 2.1 mm × 1.7 μm) column was used as a stationary phase. The gradient elution mode was used for the separation of metabolites in the samples with a flow rate of 0.4 ml/min. Five microliters filtered sample diluted in methanol was injected, and the maximum bearable pressure of the system was set to 15,000 psi. The separated metabolites were detected by the MS detector. The cone gas and nebulizer gas were set to 50 l/h and 500 l/h, respectively. The source temperature of the MS was set to 100°C while the voltage of the cone and capillary was set to 40 kV and 3.0 kV, respectively. On the basis of the analyzed m/z value, the separated metabolites were identified (Ibrahim *et al*., 2021).

2.4 Effect of extract and fractions on thrombocytopenic rats

The *in vivo* preclinical study was used to investigate antithrombocytopenic Wistar rats (150-250 g) according to standard protocol. Animals were obtained from the Central Animal House Facility of Jamia Hamdard. The study protocol was approved (Approval Number: 915) by the Institutional Animal Ethics Committee, constituted by the Committee for Control and Supervision of Experiments on Animals.

Animals were randomly distributed into seven groups (n = 6). Group I, served as control; received normal saline. Group II to Group VII received 5.0 mg/kg busulfan on days 1, 5, 10, and 15. Group II only received 5.0 mg/kg busulfan and served as toxic control while Group III to Group VII received hydroalcoholic extract (240 mg/kg), *n*-hexane fraction (25 mg/kg), DCM fraction (31 mg/kg), *n*-butanol fraction (58 mg/kg), and aqueous fraction (82 mg/kg), respectively. The dose of extract and fractions was calculated based on respective extractive values, and it is equivalent to the dose which was 500 mg/kg BW of momordica fruits (Ullah *et al*., 2012).

Based on the screening results, one potent fraction, *i.e*., DCM and mother extract were identified based on platelet count (PC). Further, blood samples of the bioactive fraction and mother extract were tested for other hematological parameters for antithrombocytopenic activity on the busulfan induced model, and other biochemical parameters such as total leukocyte count (TLC), hemoglobin count, red blood cells (RBCs), neutrophil count, packed cell volume/hematocrit (PCV/HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) were also analyzed.

The day before the start of the experiment, the PC was analyzed in all the rats. Further, platelets were counted in the experimental and busulfan treated groups on days 5, 10, 15, and 20 in an automated hematology analyzer. Blood samples were collected from the retro-orbital vein of an animal by ethylene diaminetetra acetic acid (EDTA) coated capillary tube and stored in EDTA tube after 1h of dose administration. After 1h of dosing, we have collected blood samples at the scheduled time to maintain uniformity. The collected blood samples were subjected to analysis of PC, total leukocyte count, hemoglobin count, RBC, neutrophil count, PCV/HCT, MCV, MCH, and MCHC (Wada *et al*., 2015).

2.5 Platelet function analysis

- Hematology
- Bleeding time (BT)
- Clotting time (CT)

Blood samples were collected on Day 0 and Day 7 for hematology and BT, CT). The sample was analyzed using an autoanalyzer for the following hematological parameters: platelet count (PC), total leukocyte count (TLC), bleeding time (BT), and clotting time (CT) (Lee and Lee, 2018) on days 0, 5, 10, 15 and day 20th of the experiment for selection of most active fraction.

2.6 Statistical analysis

All the experiments were performed in triplicate (n=3), and data have been expressed as mean with standard error of mean. Tukey's test was performed for the evaluation of statistical significance analysis. P values were expressed by comparing all datasets with each other at 95% confidence intervals (Kickler *et al.*, 2009).

2.7 Platelet function analysis

The PC was estimated from citrated plasma using standard kit Erbaactime and Erbaprotime made especially for CA 50 Coagulation Analyzer, Transasia (Sysmex Corporation Kobe, Japan) on days 0, 5, 10, 15, and day 20th (Dhurat and Sukesh, 2014).

3. Results

3.1 Preparation of extract and fractions

500 grams of whole fruits was extracted using ethanol 80% v/v and percentage yield was obtained, *i.e.*, 27.1% w/w. Further, these extracts were fractioned using *n*-hexane, DCM, *n*-butanol, and water, respectively. Percentage yields of the fractions were recorded such as 3.34%, 4.26%, 7.9%, and 11.11%, for *n*-hexane, DCM, n-butanol, and aqueous. During fractionation, 2.5 g of hydroalcoholic extract was lost (Figure 2).

3.2 Ultra-performance liquid chromatography-mass spectro-metry analysis of extract and fraction

Results of UPLC-MS analysis revealed good separation of metabolites and these metabolites were identified using experimental m/z values from mass bank and literature survey. Identified metabolites, retention times, m/z, and name of metabolites was summarized in Table 2.

A total of 13 metabolites were identified in the DCM fraction and extract. Out of which, three metabolites were common such as kaempferol-3-O-arabinoside, phthalamic acid, and 2-coumaric acid. There are 07 and 05 metabolites were found in the DCM fraction and mother extract, respectively. The chromatographic analysis is one of the important parameters to establish the quality of products. It is also used to reduce fraudulence in quality control analysis. Hence, these chromatographic techniques seem to be ideal for the generation of scientific data in the quality control of herbal products or botanicals. Analyzed metabolites were identified by comparing the mass with the data present in the literature (Table 1).
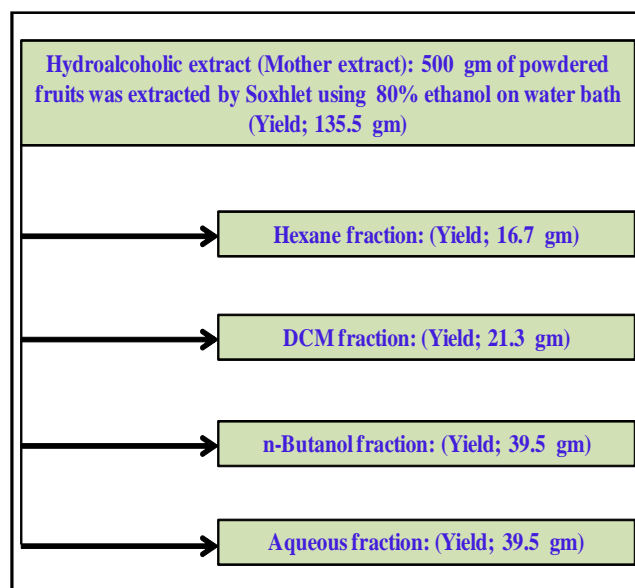

**Figure 2: Yield of mother extract and their fraction.**

Table 1: Different metabolites in DCM fraction and mother extract analyzed by UPLC-MS

| S. No.	R$_t$ (min)	m/z	Metabolite name	DCM fraction	Mother extract
1	0.21	495.34	Malvidin-3-O-glucoside	+	-
2	0.22	273.05	Phloretin	-	+
3	0.23	419.33	Kaempferol-3-O-arabinoside	+	+
4	0.28	413.267	Solasodin	+	-
5	0.32	437.34	Phloretin-2'-O-glucoside	+	-
6	0.35	205.189	Alpha-lipoic acid	+	-
7	0.52	118.086	Alpha-hydroxyisovaleric acid	-	+
8	0.94	293.20	(+)-Cinchonine	+	-
9	0.94	161.011	4-Hydroxycoumarin	+	-
10	1.00	165.0485	Phthalamic acid	+	+
11	2.00	166.0512	Alpha-hydroxyhydrocinnamic acid	-	+
12	4.65	165.0485	2-Coumaric acid	+	+
13	7.74	104.1018	Choline	-	+

3.3 The antithrombocytopenic activity of hydroalcoholic extract and its fractions

Hydroalcoholic extract and prepared fractions were administered to busulfan-treated rats. Observed results revealed that the DCM fraction and mother extract were showed better antithrombocytopenic activity as compared to other groups (Figure 3). Based on the preliminary observed results, we have considered DCM fraction and mother extract and their sham control for further evaluation of antithrombocytopenic potential and chromatographic analysis.
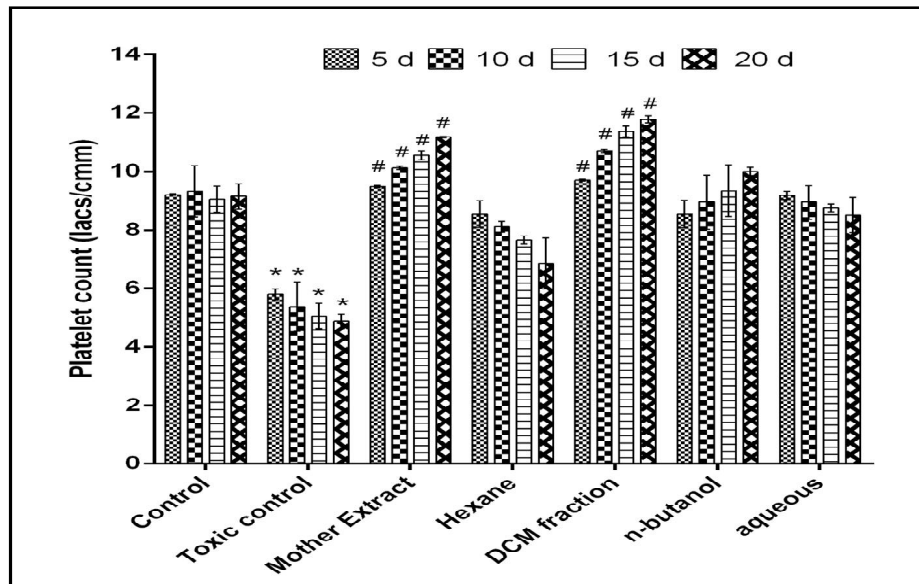
Figure 3: Effect of hydroalcoholic extract and their fraction on platelet count. Data were expressed as mean with relative standard error of mean. Significant analysis was performed with a comparison of toxic control for all days except day 5.
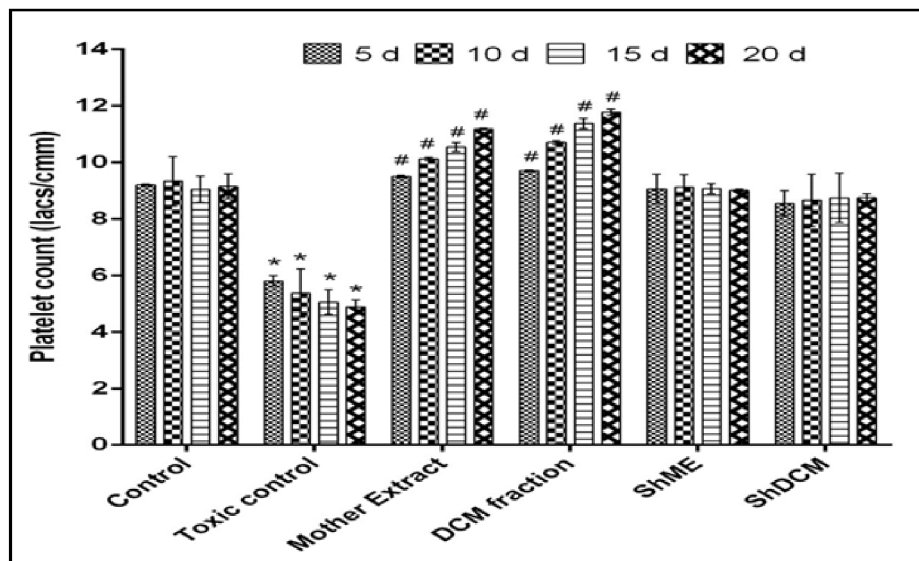
Figure 4: Effect of mother extract and their dichloromethane fraction on platelet count. Data were expressed as mean with relative standard error of mean. Significant analysis was performed with a comparison of toxic control for all days except day 5.

3.4 Platelet count

The busulfan-induced experimental model of thrombocytopenia was confirmed by the reduction of PC. After administration of busulfan to experimental rats, the level of PC was significantly decreased ($p<0.05$). Conversely, after intake of extract, the PC restore to normal as compared to an untreated rat. While *M. charantia* mother extract and DCM fraction and their respective sham control did not show any significant ($p<0.05$) change in PC as like toxic control. Contemporary, mother extract and DCM fraction treatment groups significantly ($p<0.05$) reverse the level of platelets as compared to toxic control. This effect is attributable to improved platelet redistribution from the spleen (Colli *et al.*, 2017). Among the mentioned two preparations, the DCM fraction was found to be more significant (Figure 4).

3.5 Total leukocyte count and lymphocyte count

Total leukocyte counts significantly decreased ($p < 0.05$) in the toxic group (24.2%) as compared to the normal group. The percentage of total leukocytes in the normal and sham control groups was found significantly higher ($p < 0.05$) as compared to the treatment group.

However, within 20 days of treatment with DCM fraction and mother extract, leukocyte count was significantly ($p < 0.05$) ameliorated. *M. charantia* causes no significant change ($p < 0.05$) in the percentage of lymphocyte count against busulfan-treated animals. Even the sham control groups for DCM fraction and mother extract did not produce any significant change (Table 2).

Table 2: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on total leukocyte count and lymphocyte count

Day	Parameters	Normal control	Toxic control	Mother extract	DCM fraction	Sham mother extract	Sham DCM extract
5	Leukocyte (per µl)	$10555 \pm 18.9^{**}$	8000 ± 10.2	$7300 \pm 12.6^{**}$	$9500 \pm 23.9^{**}$	$10800 \pm 4.4^{**}$	$10500 \pm 3.7^{**}$
	Lymphocyte	$87.5 \pm 0.7^{*}$	84.0 ± 1.5	$81.5 \pm 5.6^{**}$	83.5 ± 1.6^{ns}	$80.0 \pm 2.8^{*}$	83.5 ± 4.6^{ns}
10	Leukocyte (per µl)	$10405 \pm 16.0^{**}$	5950 ± 10.1	$5450 \pm 40.2^{**}$	$7950 \pm 15.4^{**}$	$10400 \pm 3.4^{**}$	$10100 \pm 3.1^{**}$
	Lymphocyte	86.5 ± 1.0^{ns}	81.5 ± 2.4	85.0 ± 2.8^{ns}	86.5 ± 0.8^{ns}	$82.5 \pm 2.7^{**}$	81.5 ± 4.7^{ns}
15	Leukocyte (per µl)	$10142 \pm 18.9^{**}$	3900 ± 10.4	$5350 \pm 8.4^{**}$	$7250 \pm 26.8^{**}$	$9900 \pm 3.1^{**}$	$11300 \pm 3.9^{**}$
	Lymphocyte	86.0 ± 1.0^{ns}	79.0 ± 1.7	79.5 ± 0.7^{ns}	$84.0 \pm 0.0^{*}$	$83.0 \pm 2.9^{**}$	79.5 ± 4.1^{ns}
20	Leukocyte (per µl)	$10400 \pm 12.1^{**}$	2800 ± 2.3	$8250 \pm 20.2^{**}$	$9200 \pm 41.8^{**}$	$9300 \pm 4.3^{**}$	$11000 \pm 3.8^{**}$
	Lymphocyte	87.5 ± 1.4^{ns}	87.5 ± 1.6	83.5 ± 2.1^{ns}	85.5 ± 4.8^{ns}	$80.0 \pm 2.6^{**}$	$76.5 \pm 4.0^{**}$

Data are expressed as mean ± SEM (n=6). Values with superscripts (*) is significantly different between toxic control vs treatment groups. The value $p<0.05$ considered significant. The symbol represents the significance level such as *$p<0.05$; **$p<0.01$ and ***$p<0.001$) and superscripts (ns) represents not significant.

Table 3: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on hemoglobin content, red blood cell, and neutrophil count

Day	Parameters	Normal control	Toxic control	Mother extract	DCM fraction	Sham mother extract	Sham DCM extract
5	RBC(Millions/mm^3)	$8.7 \pm 0.1^{**}$	7.3 ± 0.1	7.8 ± 0.2^{ns}	8.0 ± 0.2^{ns}	$8.1 \pm 0.1^{**}$	$8.86 \pm 0.2^{**}$
	Hemoglobin (g/dl)	$15.8 \pm 0.3^{**}$	13.1 ± 0.5	13.2 ± 0.4^{ns}	13.0 ± 0.1^{ns}	$14.1 \pm 0.4^{*}$	13.0 ± 0.1^{ns}
	Neutrophil (%)	$16.5 \pm 1.1^{**}$	13.0 ± 0.6	$16.6 \pm 0.5^{**}$	$14.7 \pm 0.1^{**}$	$14.3 \pm 0.4^{**}$	$13.0 \pm 0.1^{**}$
10	RBC(Millions/mm^3)	8.2 ± 0.1^{ns}	7.2 ± 1.1	6.7 ± 0.1^{ns}	7.8 ± 0.8^{ns}	$8.3 \pm 0.1^{**}$	7.35 ± 0.4^{ns}
	Hemoglobin (g/dl)	$15.6 \pm 0.3^{**}$	12.9 ± 1.4	12.0 ± 0.6^{ns}	12.2 ± 0.9^{ns}	13.5 ± 0.4^{ns}	12.1 ± 0.6^{ns}
	Neutrophil (%)	$16.1 \pm 0.3^{**}$	13.5 ± 1.4	$13.5 \pm 0.6^{**}$	13.9 ± 0.9^{ns}	13.6 ± 0.4^{ns}	12.1 ± 0.6^{ns}
15	RBC(Millions/mm^3)	8.8 ± 0.1^{ns}	7.1 ± 1.6	6.2 ± 1.1^{ns}	7.5 ± 0.4^{ns}	$8.8 \pm 1.1^{=}$	8.23 ± 0.2^{ns}
	Hemoglobin (g/dl)	$16.2 \pm 0.2^{**}$	11.8 ± 1.1	13.1 ± 0.3^{ns}	13.3 ± 2.2^{ns}	14.2 ± 1.6^{ns}	$13.1 \pm 1.1^{**}$
	Neutrophil (%)	15.5 ± 0.3^{ns}	13.0 ± 1.1	$16.0 \pm 0.40^{**}$	13.1 ± 2.3^{ns}	14.1 ± 1.7^{ns}	13.2 ± 1.1^{ns}
20	RBC(Millions/mm^3)	$8.5 \pm 0.2^{**}$	5.9 ± 0.4	6.0 ± 1.1^{ns}	$6.5 \pm 1.2^{*}$	$8.5 \pm 0.1^{**}$	$7.92 \pm 0.2^{**}$
	Hemoglobin (g/dl)	$15.9 \pm 0.4^{**}$	9.7 ± 0.7	$11.9 \pm 0.9^{*}$	$12.9 \pm 2.8^{**}$	$16.3 \pm 0.5^{**}$	$15.6 \pm 1.1^{**}$
	Neutrophil (%)	$15.5 \pm 1.1^{*}$	11.9 ± 0.8	$14.9 \pm 0.9^{**}$	12.7 ± 2.8^{ns}	$16.3 \pm 0.5^{**}$	$15.8 \pm 1.1^{**}$

Data are expressed as mean ± SEM (n = 6). Values with superscripts (*) is significantly different between toxic control vs treatment groups. The value $p<0.05$ considered significant. The symbol represents the significance level such as *$p<0.05$; **$p<0.01$ and ***$p<0.001$) and superscripts (ns) represents not significant.

3.6 Hemoglobin content, red blood cell, and neutrophil count

The rats were treated with busulfan, crude extracts of *M. charantia,* and its fractions for 20 days. On the day of 0, 5, 10, 15, and 20, blood was collected from different groups of rats. It has been observed that hemoglobin was significantly decreased ($p<0.01$) in toxic control as compared to normal control (25.17%). There was no significant difference ($p > 0.05$) observed between the normal control and sham groups. DCM fraction and mother extract of *M. charantia* showed a better effect and increase the hemoglobin against toxic control (Table 3). In the case of RBC, little change was observed in the busulfan treated groups as compared to the normal control group. On the other hand, there were no significant changes found between the normal control and sham groups (Table 3). Similar results were also observed in the case of neutrophil count. It has been observed that neutrophil count was little significantly decreased

($p < 0.01$) in toxic control as compared to normal control (19.18%). There was no significant difference ($p > 0.05$) observed between the normal control and sham groups.

3.7 Packed cell volume (PCV)

At the end of the treatment period, HCT level was significantly decreased ($p< 0.01$) in busulfan treated rats when compared to normal control; contemporarily, there was no significant difference shown between the normal control and sham groups. Busulfan caused a decrease in PCV which was reversed in rats treated with DCM fraction and mother extract of *M. charantia*. Decreased hematocrit level generally causes anemia in humans and most animals. The present study revealed that the DCM fraction of hydroalcoholic extract was identified as a bioactive fraction that restores hematocrit level close to the normal range against toxic control (Table 4).

Table 4: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on packed cell volume (PCV)

Day	Parameters	Normal control	Toxic control	Mother extract	DCM fraction	Sham mother extract	Sham DCM extract
5	MCH (pg)	33.5 ± 0.0^{ns}	28.0 ± 1.4	37.0 ± 0.7^{ns}	28.0 ± 0.7^{ns}	31.5 ± 0.7^{ns}	30.0 ± 0.7^{ns}
	MCHC(g/dl)	45.0 ± 0.3^{ns}	41.5 ± 0.7	41.5 ± 0.0^{ns}	41.0 ± 2.1^{ns}	42.5 ± 0.7^{ns}	43.0 ± 0.7^{ns}
	MCV (fl)	$76.0 \pm 0.3^{**}$	$67.5 \pm 2.1ns$	68.5 ± 2.8^{ns}	67.5 ± 2.8^{ns}	$72.0 \pm 0.0ns^{**}$	$74.0 \pm 1.1^{**}$
	PCV (%)	$59.5 \pm 1.4^{**}$	47.5 ± 0.7	48.5 ± 0.0^{ns}	48.0 ± 0.7^{ns}	$61.5 \pm 2.1^{**}$	$59.0 \pm 0.7^{**}$
10	MCH (pg)	$36.0 \pm 0.3^{**}$	27.0 ± 0.0	28.5 ± 1.4^{ns}	28.0 ± 0.7^{ns}	$32.0 \pm 1.4^{**}$	$32.5 \pm 0.6^{**}$
	MCHC(g/dl)	$46.5 \pm 0.7^{**}$	40.0 ± 0.0	41.0 ± 0.7^{ns}	41.5 ± 0.0^{ns}	43.0 ± 1.6^{ns}	42.6 ± 0.8^{ns}
	MCV (fl)	$77.0 \pm 0.3^{**}$	68.5 ± 0.7	69.5 ± 2.8^{ns}	$66.5 \pm 1.4^{*}$	$76.0 \pm 0.8^{**}$	$55.0 \pm 0.3^{**}$
	PCV (%)	$59.5 \pm 0.7^{**}$	46.5 ± 4.9	46.5 ± 1.4^{ns}	47.5 ± 2.8^{ns}	$59.0 \pm 2.1^{**}$	$56.6 \pm 1.7^{**}$
15	MCH (pg)	$36.5 \pm 0.720^{**}$	28.0 ± 1.4	28.5 ± 2.8^{ns}	28.0 ± 2.1^{ns}	30.5 ± 2.1^{ns}	31.4 ± 0.7^{ns}
	MCHC(g/dl)	$47.5 \pm 0.7^{**}$	39.5 ± 0.7	40.0 ± 0.7^{ns}	40.0 ± 2.8^{ns}	41.0 ± 1.0^{ns}	41.2 ± 0.9^{ns}
	MCV (fl)	78.5 ± 0.7^{ns}	70.5 ± 4.9	72.0 ± 7.7^{ns}	69.0 ± 0.7^{ns}	73.5 ± 0.6^{ns}	77.8 ± 0.7^{ns}
	PCV (%)	$61.5 \pm 0.7^{**}$	45.5 ± 2.1	48.0 ± 0.7^{ns}	48.0 ± 3.5^{ns}	$58.5 \pm 1.8^{**}$	$55.1 \pm 1.7^{**}$
20	MCH (pg)	$37.0 \pm 0.3^{**}$	27.0 ± 0.0	$29.5 \pm 1.4^{**}$	27.5 ± 1.4^{ns}	$35.5 \pm 1.3^{**}$	30.5 ± 0.8^{ns}
	MCHC(g/dl)	$47.5 \pm 1.4^{**}$	38.0 ± 1.4	$41.5 \pm 0.0^{**}$	$40.0 \pm 0.7^{**}$	39.0 ± 0.9^{ns}	$44.8 \pm 0.6^{**}$
	MCV (fl)	77.5 ± 0.7^{ns}	72.5 ± 3.5	74.0 ± 4.9^{ns}	$67.0 \pm 3.5^{**}$	75.5 ± 0.7^{ns}	78.5 ± 1.4^{ns}
	PCV (%)	$57.5 \pm 0.7^{**}$	42.5 ± 0.7	47.5 ± 4.2^{ns}	47.0 ± 7.7^{ns}	$57.0 \pm 1.9^{**}$	$57.2 \pm 1.6^{**}$

Data are expressed as mean ± SEM (n = 6). Values with superscripts (*) is significantly different between toxic control vs treatment groups. The value $p<0.05$ considered significant. The symbol represents the significance level such as *$p<0.05$; **$p<0.01$ and ***$p<0.001$) and superscripts (ns) represents not significant.

3.8 Mean corpuscular volume (MCV)

MCV is directly related to RBC and increases or decreases following the average red cell size. This study revealed that MCV significantly decreased ($p<0.01$) in toxic control as compared to normal control. Our most intriguing findings are revealed that bioactivity-guided fractions from the hydroalcoholic extract. Table 4 clearly shows the percentage changes in MCV among all the groups. DCM fraction and mother extract showed better results as compared to other fractions. Further, this result showed the ameliorative potential of *M. charantia* to normalize the number of MCV.

3.9 Mean corpuscular hemoglobin (MCH)

The value of MCH depletion and elevation were analyzed in all the experimental groups. The MCH levels were not affected in the toxic group and were found almost similar to the normal control group. Interestingly, there was no significant difference ($p>0.05$) was observed between the normal control group and sham groups. However, *M. charantia* DCM fraction and mother extract possessed quite similar effects against toxic control (Table 4).

3.10 Mean corpuscular hemoglobin concentration (MCHC)

The value of MCHC depletion and MCHC elevation were investigated in different experimental groups. No significant changes ($p > 0.05$) in MCHC were observed in busulfan-treated rats as compared to normal control rats. Contemporarily, there were no significant changes found between the normal control and sham groups; hence, it was clear that *M. charantia* does not produce any toxicity. *M. charantia* DCM fraction and mother extract possessed almost similar ameliorative.The observed results are presentedin Table 4.

3.11 Platelet function analysis

Effect of *M. charantia,* bioactive guided fractions, and metabolite enriched fractions on BT and CT in albino Wistar rat was found significant. Preliminary screening data were presented in Table 5 while final observation was presented in Tables 6 and 7 for CT and BT, respectively.

Table 5: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on bleeding (BT) and clotting time (CT)

Groups	Bleeding time (s) (Mean ± SEM)		Clotting time (s) (Mean ± SEM)	
	0 Day	7 Day	0 Day	7 Day
Normal control	00.216 ± 6.52	237.16 ± 5.87	165.25 ± 4.25	172.23 ± 4.29
Mother extract	206.83 ± 5.59^{ns}	305.66 ± 6.21^{ns}	165.03 ± 4.89^{ns}	190.14 ± 6.32^{ns}
Hexane fraction	280.50 ± 11.61^{ns}	278.50 ± 11.18^{ns}	170.33 ± 04.61^{ns}	172.66 ± 05.20^{ns}
DCM fraction	252.00 ± 6.11^{ns}	310.00 ± 6.99^{ns}	157.45 ± 5.32^{ns}	175.87 ± 5.78^{ns}
n-butanol	261.00 ± 10.54^{ns}	235.00 ± 9.2^{ns}	213.05 ± 7.4^{ns}	196.05 ± 5.68^{ns}
Aqueous fraction	261.00 ± 9.5^{ns}	243.00 ± 8.21^{ns}	162.00 ± 5.90^{ns}	191.83 ± 6.2^{ns}

Table 6: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on clotting time (CT)

Groups	Clotting time (s) (Mean ± SEM)				
	Day 0	Day 5	Day 10	Day 15	Day 20
Normal control	165.25 ± 4.25^{ns}	$172.23 \pm 4.29^{**}$	$170.30 \pm 3.17^{**}$	168.16 ± 5.12^{ns}	162.83 ± 4.87^{ns}
Toxic control	166.85 ± 5.68	211.34 ± 6.54	198.56 ± 7.25	177.66 ± 5.45	165.50 ± 4.56
Mother extract	165.03 ± 4.89^{ns}	219.14 ± 6.32^{ns}	201.02 ± 8.54^{ns}	185.05 ± 5.99^{ns}	157.00 ± 5.87^{ns}
DCM extract	163.54 ± 6.11^{ns}	$187.45 \pm 4.58^{*}$	$178.54 \pm 5.54^{*}$	161.33 ± 4.75^{ns}	$148.33 \pm 4.85^{*}$
Sham mother extract	168.25 ± 5.55^{ns}	$185.47 \pm 4.67^{*}$	$164.31 \pm 5.19^{*}$	$153.05 \pm 4.23^{**}$	$149.16 \pm 5.21^{*}$
Sham DCM extract	157.45 ± 5.32^{ns}	199.87 ± 5.78^{ns}	191.26 ± 8.37^{ns}	181.05 ± 4.86^{ns}	$149.66 \pm 4.23^{*}$

Table 7: Effect of DCM fraction, mother extract and their respective sham groups of *M. charantia* on bleeding time (BT)

Groups	Bleeding time (s) (Mean ± SEM)				
	Day 0	Day 5	Day 10	Day 15	Day 20
Normal control	216.00 ± 6.52^{ns}	$237.16 \pm 5.87^{*}$	$221.50 \pm 6.25^{*}$	234.33 ± 6.58^{ns}	239.5 ± 7.25^{ns}
Toxic control	227.83 ± 5.45^{ns}	295.56 ± 5.48	281.15 ± 6.25	270.00 ± 6.77	242.16 ± 6.45
Mother extract	206.83 ± 5.59^{ns}	305.66 ± 6.21^{ns}	285.54 ± 7.80^{ns}	244.00 ± 5.46^{ns}	216.33 ± 7.41^{ns}
DCM extract	246.83 ± 6.32^{ns}	281.16 ± 6.32^{ns}	263.43 ± 7.24^{ns}	255.16 ± 6.32^{ns}	228.8 ± 5.41^{ns}
Sham Mother extract	261.83 ± 6.47^{ns}	303.66 ± 7.47^{ns}	273.89 ± 8.23^{ns}	229.83 ± 4.38^{ns}	214 ± 7.89^{ns}
Sham DCM extract	252.00 ± 6.11^{ns}	300.00 ± 6.99^{ns}	278.00 ± 7.13^{ns}	252.83 ± 5.47^{ns}	228.74 ± 7.99^{ns}

4. Discussion

According to previously cited literature, *M.charantia* was investigated for its antithrombocytopenic effect but the proper scientific evaluation was not done. To date, no such studies reported for metabolic characterization of *M. charantia* and its action mechanism either clinically or preclinically. Keeping in mind, we strive to generate scientific data regarding its antithrombocytopenic efficacy and also enabled the bioactive metabolites using UPLC-MS.

Thrombocytopenia was successfully developed in Wistar rats by administration of busulfan induced. Moreover, the efficacy of DCM and mother extract of *M. charantia* was evaluated on busulfan-induced thrombocytopenic rats models. The present investigation suggested that DCM fraction impressively enhance platelets counts as compared to other fraction.

Interestingly, DCM fraction and mother extract possess significant effect ($p < 0.05$) against busulfan-induced groups and ameliorated the

hematological parameters like a platelet, total lymphocyte, neutrophil count, leukocyte, hemoglobin, RBC, MCH, PCV/HCT, MCV, and MCHC. Restoration of the above-mentioned parameters was found more impressive in the case of the DCM fraction of *M. charantia*.

Presented results disclosed that some of the bioactive metabolites present in *M. charantia* can improve the hematological parameters in busulfan-induced thrombocytopenic rats. Bioactive metabolites, available in the fruits of *M. charantia,* avert the destruction of platelet in blood and ultimately improve the life of platelet in blood circulation. On the basis of the current studies, it can be concluded that *M. charantia* might be a promising platelet improving remedy or antithrombocytopenic agent. Cited literature reported that *M. charantia* alcoholic extract possesses an inhibitory effect on DENV-1.

Thrombocytopenia is a common clinical manifestation in which the PC counts were dropped from normal counts ($< 40,000$ platelets/µl) and might be reached to lower than $< 40,000$ platelets/µl of the blood sample (Ahmad *et al.,* 2019)**.** Both DCM fraction and mother extracts of *M. charantia* fruits showed excellent efficacy against viral infection, and these fractions and extracts were capable to reverse the blood count near to normal (Machado *et al.,* 2018). Earlier studies reported that, *M. charantia* enhanced PC in the rat (Arollado, 2013).

M. charantia has been extensively utilized in traditional medicine to improve thrombocyte count in dengue viral infection (Anon, 2013)**.** Physiologically, cytokine modulates the production of thrombocyte levels. Therefore, a diminished level of cytokine may reduce platelet production and might lead to the thrombocytopenic condition. Some bioactive compounds stimulate the production of cytokine, and this biological exploration of thrombopoietin justifies the traditional medicinal claims for the pharmacological action of *M. charantia* in thrombocytopenia.

UPLC-MS qualitative analysis of different extracts and fractions indicate that *M. charantia* is enriched with bioactive metabolites. Most of the metabolites identified in the extract or fraction are responsible for pharmacological activities. Chromatographic studies are one of the prime approaches to evaluate the quality of herbal formulation or food supplements. In the current present study, UPLC-MS was performed to identify and authenticate the fraction and extract of *M. charantia* fruits to establish the quality. Bioactive metabolites present in the plant having good antioxidant potential, especially polyphenols, are capable to improve platelet production (Djati, 2017)**.** Our study and cited literature suggested that *M. charantia* potentially increases PC level (Blache and Bontoux, 1988). The presence of many bioactive metabolites strongly supports the traditional system of medicine claimed by healers about *M. charantia*. Based on the present finding, *M. charantia* excellently improves the antithrombocytopenic activity in a busulfan-induced thrombocytopenic rat model.

5. Conclusion

The standardized mother extract of *M. charantia* and its DCM fraction significantly ameliorates PC in thrombocytopenic rats. This is the first study that highlights the antithrombocytopenic potential of *M. charantia* scientifically. Hence, extensive work is required to identify the responsible bioactive compounds in *M. charantia* for the anti-thrombocytopenic activity.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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